Hereditary Postprandial Hypertriglyceridemic Rabbit Exhibits Insulin Resistance and Central Obesity
A Novel Model of Metabolic Syndrome

Takashi Kawai, Tsunekata Ito, Kazuo Ohwada, Yasuko Mera, Mutsuyoshi Matsushita, Hitonobu Tomoike

Objective—We have established a hereditary postprandial hypertriglyceridemic (PHT) rabbit. The present study was designed to define whether this rabbit model represents both insulin resistance and central obesity.

Methods and Results—Body weight, abdominal circumference, visceral fat weight, and glucose tolerance were compared between PHT and Japanese white (JW) rabbit. Plasma levels of triglycerides (TG), total cholesterol (TC), glucose, and insulin were measured before and after feeding. Abdominal circumference of PHT rabbit was larger than that of JW rabbit, with no difference in body mass index. Visceral fat accumulation was noted as obvious in mesenterium, retroperitoneal space, and epididymal area. Plasma TG and TC levels were high preprandially and markedly increased postprandially in PHT rabbit compared with JW rabbit. Although plasma glucose levels were comparable in both groups, plasma insulin levels were elevated in PHT rabbit. Glucose tolerance tests indicated that plasma insulin levels in PHT rabbit were consistently higher than in JW rabbit. A positive correlation was observed between plasma insulin levels and visceral fat weight in PHT rabbit.

Conclusions—PHT rabbit shows insulin resistance along with central obesity. PHT rabbit will serve as a model for elucidating genetic predisposition and pathophysiology in metabolic syndrome. (Arterioscler Thromb Vasc Biol. 2006; 26:2752-2757.)

Key Words: central obesity ■ heritable model ■ insulin resistance ■ metabolic syndrome ■ postprandial hypertriglyceridemia

Metabolic syndrome has recently gained attention as an underlying milieu of atherosclerosis.1–3 Recent US statistics show that metabolic syndrome affects ≈1 in 4 adults and that the incidence of metabolic syndrome is sharply increasing with the growing population of obese individuals with visceral adiposity due partly to overeating, lack of physical exercise, and a high-fat diet.4 In light of its clinical significance, development of therapeutic remedies for metabolic syndrome is of prime importance and therefore the establishment of an animal model is urgently required.

Metabolic syndrome is characterized by a clustering of several cardiovascular risk factors in a single individual, including dyslipidemia, centrally distributed adiposity, hypertension, and syndromes of glucose intolerance. Although multiple disorders were partly modeled by glucose intolerance in heritable rodent models,5,6 lipid metabolism differs considerably between humans and rodents (eg, lack of cholesteryl ester transfer protein, differences in the apoB synthesis pathway).7–9 To apply contemporary approaches using genetically engineered mice or translucent zebrafish, genetic phenotypes of metabolic syndrome remain too complex.

An animal model of familiar hypercholesterolemia is unique in Watanabe heritable hyperlipidemic (WHHL) rabbit, because it closely resembles cholesterol metabolism in humans. We successfully segregated a hypertriglyceridemic type of WHHL (WHHL-TGH) rabbit, and then established a postprandial hypertriglyceridemic (PHT) rabbit from a cross between a normal Japanese white (JW) rabbit and a WHHL-TGH rabbit.10 The present study was aimed to clarify the levels of central obesity and glucose intolerance in our newly established animal model of human disease.

Materials and Methods

Animals

Male 8- to 16-month-old PHT rabbits (number of rabbits: 19) and 10- to 17-month-old JW rabbits (number of rabbits: 6; Shiraishi Laboratory Animals, Tokyo, Japan) were housed in an animal room maintained at 22±2°C with 40% to 60% RH and a light period from 6:00 to 18:00 in the Laboratory Animal Center of Yamagata

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From Laboratory Animal Center (T.K., T.I., K.O.), Yamagata University School of Medicine, Yamagata-shi, Yamagata, Japan; Japan Tobacco Inc (T.K., Y.M., M.M.), Central Pharmaceutical Research Institute, Biological/Pharmacological Research Laboratories, Murasaki-cho, Takatsuki, Osaka, Japan; National Cardiovascular Center (H.T.), Fujishiro-dai, Suita, Osaka, Japan.

Correspondence to Takashi Kawai, Japan Tobacco Inc, Central Pharmaceutical Research Institute, Biological/Pharmacological Research Laboratories, 1-1 Murasaki-cho, Takatsuki, Osaka 569-1125, Japan. E-mail takashi.kawai@ims.jti.co.jp

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University School of Medicine. Rabbits were fed a standard diet (120 g per rabbit per day; Labo R-Grower, Nihonnosan, Yokohama, Japan), and provided with water ad libitum. Body weights were measured, and under a mixed anesthesia of ketamine (35 mg/kg, intramuscularly) and xylazine (5 mg/kg, i.m.), body length, defined as the distance from the tip of the nose to the anus, and abdominal circumference were also measured. At 24 hours after food supply, animals were exsanguinated under pentobarbital anesthesia (30 mg/kg, intravenous). Mesenteric fat, retroperitoneal fat, and epididymal fat were removed and weighed at necropsy.

Measurement of Blood Pressure and Heart Rate
Measurement of blood pressure used male 14- to 27-month-old PHT rabbits (number of rabbits: 6) and 21-month-old JW rabbits (number of rabbits: 4) was performed in accordance with the method of Kuwahara et al.11 Unanesthetized rabbits were stayed comfortably in holder. A cuff was placed around the crus of right limb. Systolic blood pressure, mean blood pressure, and heart rate levels in rabbits were measured using an oscillographic device (Sofron BP-98E; Sofron, Tokyo, Japan). Diastolic blood pressure was calculated from systolic blood pressure and mean blood pressure levels.

Measurement of Plasma Triglycerides, Total Cholesterol, Glucose, Insulin, and Nonesterified Fatty Acid Levels
Blood samples were collected via the auricular artery. Samples were stored on ice and centrifuged (1000g, 15 minutes, 4°C) to obtain plasma. Plasma triglycerides (TG), total cholesterol (TC), and glucose levels were measured using TG, TC, and glucose assay kits (Roche Diagnostics, Tokyo, Japan), respectively, and an autoanalyzer (HITACHI-7170S; Hitachi, Tokyo, Japan). Cholesterol and triglyceride profiles in plasma lipoproteins were analyzed using a dual detection high-performance liquid chromatography system with 2 TSKgel LipopropakXL columns (300×7.8-mm; Tosoh, Japan) connected in tandem (Skylight Biotech Inc, Akita, Japan).12,13 Plasma insulin level was measured using an enzyme-linked immunosorbent assay kit (Morinaga Institute of Biological Science, Yokohama, Japan). Plasma nonesterified fatty acid (NEFA) level was measured using an NEFA assay kit (Wako, Osaka, Japan).

Oral Glucose Tolerance Test and Intravenous Glucose Tolerance Test
Oral glucose tolerance test (OGTT) was performed in accordance with the method of Baukje de Roos et al.14 After a fasting period of 12 hours, a 50% glucose solution was orally administered to the animals at a dose of 1.5 g/kg. Blood samples were collected via the auricular artery before and 15, 30, 45, 60, 90, 120, and 240 minutes after glucose loading. Intravenous glucose tolerance test (IVGTT) was performed in accordance with the methods of Koike et al and Shimoi et al.15,16 After a fasting period of 12 hours, a 50% glucose solution was i.v. administered to the animals at a dose of 0.6 g/kg. Blood samples were collected via the auricular artery before and 5, 10, 15, 30, 45, 60, and 120 minutes after glucose loading. Samples were stored on ice and centrifuged (1000g, 15 minutes, 4°C) to obtain plasma. Plasma glucose, insulin, and NEFA levels were measured in the manner described. Insulin resistance (IR) index was calculated from plasma glucose and insulin levels.

Statistical Analysis
Results are presented as means±standard error of mean (SEM). Significance of differences between JW and PHT rabbits was evaluated using an F test, followed by Student t test or Aspin-Welch t test. The correlation between visceral fat weight in individual JW or PHT rabbits and plasma insulin levels was tested by Pearson correlation analysis. A value of P<0.05 was considered significant.

Results
Morphological Features, Visceral Fat Accumulation, and Blood Pressure
PHT rabbits (=1 year old) were smaller than JW rabbits (Figure 1A and 1B), with body weight and body length of PHT rabbits significantly less than JW rabbits (3.20±0.04 kg and 501±2.1 mm versus 3.57±0.08 kg and 539±3.7 mm, respectively; Table). Body mass index (BMI) was calculated as an index of obesity. Although there was no difference in BMI between the 2 groups, the abdominal circumference of PHT rabbits was larger than that of JW rabbits and abdominal circumference/body length ratio was significantly higher (0.75±0.01 versus 0.66±0.01; Table). In addition, a marked visceral fat accumulation was noted in regions of mesenterium, retroperitoneum, and epididymis of PHT rabbits (Figure 1C, 1D). Mesenteric fat/body weight, retroperitoneal fat/body weight, and epididymal fat/body weight ratios in PHT rabbits were significantly larger than those in JW rabbits (26.5±2.5 g/kg body weight [BW], 27.8±4.2 g/kg BW and 1.53±0.07 g/kg BW versus 13.5±3.5 g/kg BW, 12.0±4.5 g/kg BW and 0.64±0.06 g/kg BW, respectively; Figure 1E, 1F, and 1G).

Diastolic blood pressure in PHT rabbits were significantly higher than JW rabbits (80±3 mm Hg versus 69±4 mm Hg; Table). Systolic blood pressure and mean
Comparison of Morphological Features and Blood Pressure in PHT Rabbits and JW Rabbits

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<thead>
<tr>
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<th>JW Rabbit</th>
<th>PHT Rabbit</th>
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<tr>
<td>Body weight, kg</td>
<td>3.57 ± 0.08</td>
<td>3.20 ± 0.04†</td>
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<tr>
<td>Body length, mm</td>
<td>539 ± 3.7</td>
<td>501 ± 2.1†</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>12.3 ± 0.33</td>
<td>12.8 ± 0.11</td>
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<tr>
<td>Abdominal circumference, mm</td>
<td>357 ± 3.3</td>
<td>376 ± 2.6†</td>
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<tr>
<td>Abdominal circumference/body length ratio, mm/mm</td>
<td>0.66 ± 0.01</td>
<td>0.75 ± 0.01†</td>
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<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>112 ± 7</td>
<td>119 ± 4</td>
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<tr>
<td>Mean blood pressure, mm Hg</td>
<td>83 ± 5</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>69 ± 4</td>
<td>80 ± 3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>219 ± 12</td>
<td>247 ± 6*</td>
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Comparison of body weight, body length, body mass index, abdominal circumference, and abdominal circumference/body length ratio in 13- to 17-month-old JW and 10- to 16-month-old PHT rabbits. Comparison of systolic blood pressure, mean blood pressure, diastolic blood pressure, and heart rate in 21-month-old JW and 14- to 27-month-old PHT rabbits. Data are expressed as mean ± SEM (JW: n = 6; PHT: n = 19; morphological features, JW: n = 4; PHT: n = 6; blood pressure).

*P < 0.05, †P < 0.01 vs JW rabbit (F and t tests).

blood pressure in PHT rabbits were higher than JW rabbits, not significant (119 ± 4 mm Hg and 93 ± 3 mm Hg versus 112 ± 7 mm Hg and 83 ± 5 mm Hg, respectively; Table). Heart rate in PHT rabbits was significantly higher than JW rabbits (247 ± 6 bpm versus 219 ± 12 bpm; Table).

Postprandial Plasma TG, TC, Glucose, Insulin, and Fasting NEFA Levels

Preprandial plasma TG levels were significantly higher in PHT rabbits than in JW rabbits (4.55 ± 1.32 mmol/L versus 0.40 ± 0.09 mmol/L). After 15 hours of continuously available feed, plasma TG levels were markedly elevated to 15.9 ± 2.7 mmol/L in PHT rabbits, compared with 0.71 ± 0.01 mmol/L in JW rabbits (Figure 2A). Chylomicron (CM), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels in TG were higher in PHT rabbits than JW rabbits. Marked elevations of CM and VLDL TG levels were noted in PHT rabbits (Figure 2C).

Preprandial plasma TC levels were also significantly higher in PHT rabbits than in JW rabbits (1.95 ± 0.37 mmol/L versus 0.56 ± 0.04 mmol/L). After 15 hours of continuously available feed, plasma TC levels were markedly elevated to 2.76 ± 0.30 mmol/L in PHT rabbits, but unchanged in JW rabbits (0.57 ± 0.03 mmol/L) (Figure 2B). CM, VLDL, and LDL cholesterol levels were higher in PHT rabbits than in JW rabbits; however, HDL cholesterol levels were not different between PHT and JW rabbits (Figure 2D).

Plasma glucose levels in PHT rabbits were significantly low at postprandial 2, 12, 15, and 18 hours compared with JW rabbits (Figure 3A). Preprandial insulin levels were significantly higher in PHT rabbits than in JW rabbits (224 ± 50 pmol/L versus 27.4 ± 6.6 pmol/L). Plasma insulin levels in PHT rabbits increased to 508 ± 125 pmol/L at postprandial 6 hours, and remained high thereafter (Figure 3B). Preprandial plasma NEFA levels were significantly higher in PHT rabbits than in JW rabbits (0.48 ± 0.04 mEq/L versus 0.33 ± 0.04 mEq/L; Figure 3C).

OGTT and IVGTT

Plasma glucose levels before glucose loading were similar in PHT and JW rabbits. Oral glucose loading increased plasma glucose levels transiently in both PHT and JW rabbits; however, slightly higher levels of plasma content trajectory in PHT than in JW rabbits were not statistically significant.
Plasma insulin levels before glucose loading were significantly higher in PHT rabbits than in JW rabbits (256 ± 42 pmol/L versus 30.2 ± 6.3 pmol/L). Plasma insulin after glucose loading increased enormously in PHT rabbits to 696 ± 89 pmol/L at 45 minutes, and remained at higher levels in PHT than in JW rabbits. Plasma NEFA levels before glucose loading were significantly higher in PHT rabbits than in JW rabbits (1.14 ± 0.08 mEq/L versus 0.65 ± 0.07 mEq/L). After loading, levels in PHT rabbits were consistently and significantly higher than in JW rabbits. IR index, based on insulin area under the curve (AUC) and glucose AUC, was significantly higher in PHT rabbits than in JW rabbits (Figure 4A).

IVGTT results showed that plasma glucose levels before glucose loading were similar (~6.7 mmol/L) in PHT and JW rabbits. Plasma glucose levels 5 minutes after glucose loading elevated to ~24 mmol/L in both PHT and JW rabbits. At 60 minutes after loading, plasma glucose levels were significantly higher in PHT rabbits than in JW rabbits, decreasing to 9.4 ± 0.6 mmol/L and 7.2 ± 0.3 mmol/L in PHT and JW rabbits, respectively. Plasma insulin levels before glucose loading were significantly higher in PHT rabbits than in JW rabbits (343 ± 44 pmol/L versus 42.7 ± 17.1 pmol/L). At 5 minutes after glucose loading, plasma insulin levels in PHT rabbits increased to 860 ± 138 pmol/L, with subsequent levels also significantly higher than in JW rabbits. Plasma NEFA levels before glucose loading were higher in PHT rabbits than in JW rabbits (0.83 ± 0.31 mEq/L versus 0.18 ± 0.03 mEq/L). After glucose loading, levels were also consistently higher than in JW rabbits, although the difference was not significant. IR index was significantly higher in PHT rabbits than in JW rabbits (Figure 4B). A significant positive correlation was observed in IR indexes between OGTT and IVGTT in PHT rabbits (data not shown).

**Correlation Between Visceral Fat Weight and Plasma Insulin Level**

There was a significant positive correlation between plasma fasting insulin levels in PHT rabbits and visceral fat weight (the sum of mesenteric, retroperitoneal and epididymal fat weight; Figure 5A). A positive correlation was also observed between plasma insulin AUC derived from IVGTT and visceral fat weight in PHT rabbits (P = 0.068, Figure 5B).

**Discussion**

The primary finding of this study was that this heritable model of PHT rabbit exhibits IR and central obesity. This is
the first evidence that the naturally segregated animal model represents the clinical manifestations of metabolic syndrome. Our results underscore the importance of exploiting a pure-bred model to study the polygenic disease, so that results might be ultimately translated to preventive as well as therapeutic strategies.

With regard to lipid metabolism, rabbits have several advantages over other species: rabbits have higher levels of apoB-containing lipoproteins than mice or rats, the lipoprotein profile is more like that of humans, and patterns of hepatic apoB100 and intestinal apoB48 resemble those of humans. Furthermore, unlike mice and rats, rabbits have cholesteryl ester transfer protein similar to humans.7–9 These metabolic characteristics inherent in rabbits suggest the feasibility of an animal model of human disease with complex manifestations such as metabolic syndrome. We have segregated a heritable rabbit model (PHT rabbit) that exhibits postprandial hypertriglyceridemia, in which extremely high plasma levels of both cholesterol and triglycerides are observed in a WHHL-TGH line disappeared after hybridizing with JW rabbit, a wild type. Namely, PHT rabbit appeared as the second generation in crossing between the heterozygous JW rabbit and WHHL-TGH rabbits. Interestingly, rabbits with hypercholesterolemia were not obtained, in spite of crossing over several generations in PHT rabbit. Because PHT rabbit is free of genetic hypercholesterolemia, the present rabbit model is a new line quite different from the genealogy of WHHL rabbits.

An enlarged abdominal circumference or a visceral adiposity has been classified in World Health Organization (WHO) and National Cholesterol Education Program–Adult Treatment Panel III (NCEP-ATP III) as a physical characteristic specific to metabolic syndrome.17,18 In PHT rabbit, enlarged abdominal circumference and marked visceral fat accumulation were noted (Table; Figure 1). Accordingly, PHT rabbit is a model that closely resembles a type of central obesity in humans. By molecular biological approaches to adipose tissue, it was demonstrated that abnormal secretion of adipocytokines from adipose tissue are closely associated with the progress of metabolic syndrome.19,20 Increase in the secretion of tumor necrosis factor-α (TNF-α), plasminogen activator Inhibitor-1 (PAI-1) from enlarged adipocyte are highly related to the development of obese and IR.21–24 In our observation, adipocytes in PHT rabbit were larger than in JW rabbit histopathologically (data not shown). Therefore, it was considered that PHT rabbit has an abnormal secretion of adipocytokines from adipose tissue.

Impaired glucose tolerance (IGT), but not impaired fasting glucose, is one of features of the present PHT rabbit. IR in the present model was related to a marked hyperinsulinemia but not hyperglycemia, demonstrated both before and after glucose loading. Higher levels of fasting NEFA in PHT rabbit also support the presence of insulin resistant state (Figure 3C), which represents an attenuation of the antilipolytic effect of insulin in adipose tissue.25,26 Interestingly, plasma glucose levels during the glucose loading were approximately similar for up to 30 minutes in trend between PHT and JW rabbits. Thus, the IR of PHT rabbit was derived from hyperinsulinemia, but not hyperglycemia (Figures 3, 4). It is well known that hyperinsulinemia has been detected in prediabetic subjects as early as 1 to 2 decades before clinical onset. Accordingly, PHT rabbit is an animal model of IGT and IR, but not diabetes mellitus.

The standard OGTT showed hyperinsulinemia and IGT in PHT rabbits. IVGTT, performed to assess both the β-cell secretory capacity and peripheral glucose uptake, showed both the glucose-induced augmented secretion of insulin and peripheral IR (Figure 4). The magnitude of the insulin response to the amount of glucose available was augmented in OGTT, which supports the importance of the mode of glucose entry and the simultaneous release of gut peptides.

It has been reported that there is a positive correlation between accumulation of visceral fat and fasting plasma insulin levels.27 As strong correlations were seen between visceral fat weight and fasting plasma insulin levels as well as plasma insulin AUC in GTT in the present model (Figure 5), central obesity might exaggerate IR and augment NEFA levels (Figures 3, 4). Thus, PHT rabbit is a very useful model for characterizing the relationship between the severities of IR or IGT and visceral fat accumulation.

Decreased lipoprotein lipase caused by impaired catabolism of CM and VLDL, results in high TG levels.28,29 In preliminary data, lipoprotein lipase activity of PHT rabbit was lower than that of JW rabbit (data not shown), marked increase of postprandial CM and VLDL TG in PHT rabbits may be strongly caused by decreasing lipoprotein lipase activity. Plasma NEFA levels were significantly higher in PHT rabbits than in JW rabbits, and preprandial plasma TG levels were also significantly higher in PHT rabbits (Figures 2, 3). Free fatty acids (FFAs) related to high TG levels or visceral fat also have been known to induce peripheral IR.20,26,30

Subjects with metabolic syndrome are frequently associated with hypertension, and potentially at high risk for atherosclerosis. In present study, blood pressure in PHT rabbit tended to be high compared with JW rabbit (Table). In addition, it has been reported that PHT rabbit has vascular dysfunction derived from vascular endothelial cells.31 PHT rabbit may be a very useful model to investigate the influence of metabolic syndrome on vascular dysfunction.

In conclusion, PHT rabbit, a novel model of metabolic syndrome, has not only disorders of lipid metabolism but also impaired glucose tolerance and IR, partly caused by visceral adiposity. The pathophysiology of the PHT rabbit coincides well with the current concept of metabolic syndrome, which contends that visceral fat accumulation is the fundamental disorder, and that hyperinsulinemia, glucose intolerance, and lipid metabolism disorders are intimately related to the evolution of IR. As the metabolic pattern for rabbits resembles that in humans, PHT rabbits have clinical usefulness that is lacking in other animal models.
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Disclosures

None.

References

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