Hypertriglyceridemia in Watanabe Heritable Hyperlipidemic Rabbits Was Associated with Increased Production and Reduced Catabolism of Very-Low-Density Lipoproteins

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\textbf{Introduction}

There are growing lines of evidence showing that hypertriglyceridemia is a potential risk factor for coronary artery disease \cite{1–4}. It has been demonstrated that a therapeutic lowering of plasma triglycerides (TG) reduces the occurrence of coronary artery disease \cite{5}. Watanabe heritable hyperlipidemic (WHHL) rabbits were established 30 years ago by Dr. Watanabe at Kobe University, Japan, and have been used as an excellent animal model for human familial hypercholesterolemia \cite{6}. WHHL rabbits show spontaneous hypercholesterolemia and atherosclerosis due to a genetic defect of low-density lipoprotein (LDL) receptors \cite{6–8}. In our facility, we bred WHHL rabbits obtained from Kobe University and observed that there was a large range of plasma levels of TG (ranging from 150 to 1,050 mg/dl) among WHHL descendants. After selected inbreeding, we segregated 2 WHHL colonies; one with plasma levels of TG \textsuperscript{1}500 mg/dl (designated as TGH-WHHL) and another with relatively lower plasma levels of TG (\textsuperscript{1}250 mg/dl), designated as TGL-WHHL rabbits \cite{9, 10}. These 2 kinds of WHHL rabbits allowed us to investigate the possible mechanisms of hypertriglyceridemia and its relationship with glucose metabolism and insulin sensitivity. We found that severe hypertriglyceridemia in

\textbf{Key Words}

Hypertriglyceridemia • Watanabe heritable hyperlipidemic rabbits • Lipoprotein lipase • Hypercholesterolemia

\textbf{Abstract}

Watanabe heritable hyperlipidemic (WHHL) rabbit is an animal model for human familial hypercholesterolemia. Recently, we segregated a new mutant of WHHL rabbits with plasma levels of triglycerides (TG) \textsuperscript{1}500 mg/dl (designated as TGH-WHHL). To investigate the underlying mechanisms for hypertriglyceridemia, we compared TGH-WHHL with WHHL rabbits with lower plasma TG levels (\textsuperscript{1}250 mg/dl, designated as TGL-WHHL). A Triton WR-1339 injection experiment revealed that TGH-WHHL rabbits had increased secretion and decreased clearance of TG-rich lipoproteins. Furthermore, TGH-WHHL rabbits had lower a post-heparin activity of lipoprotein lipase and a higher cholesterol ester transfer protein activity than TGL-WHHL rabbits. Cultured hepatocytes isolated from TGH-WHHL rabbits showed a higher secretion rate of TG and cholesterol than those of TGL-WHHL rabbits. In addition, TGH-WHHL rabbits exhibited marked insulin resistance. These data suggest that hypertriglyceridemia exhibited by WHHL rabbits is caused by both increased production and impaired catabolism of TG-rich lipoproteins and associated with insulin resistance.
TG Secretion and Catabolic Rates in vivo

Rabbits, 12 month old, were used after overnight fasting. Blood samples were centrifuged at 2,000 rpm for 10 min, at 4°C. The plasma TG and total cholesterol (TC) levels were measured by Abbott Vision kits (Dinabot, Ltd., Tokyo, Japan) as described previously [11]. Rabbits used for this study were male and 4, 7 and 12 months old. Age- and sex-matched Japanese white (JW) rabbits from Kumagai (Saitama, Japan) were used as a normal control. The 3 groups of rabbits were maintained individually in a controlled environment with free access to water and a chow diet (Labo-R-Grower, Nihon Nosan Kogyo, Ltd., Tokyo, Japan). All animal experiments were performed with the approval of the Animal Care Committee of the University of Yamagata and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Analysis of Plasma Lipids and Enzyme Activity

Blood was collected from a marginal ear vein after overnight fasting. Blood samples were centrifuged at 2,000 rpm for 10 min, at 4°C. The plasma TG and total cholesterol (TC) levels were measured by Abbott Vision kits (Dinabot, Ltd., Tokyo, Japan) [12]. Post-heparin enzymatic activity of lipoprotein lipase (LPL) and hepatic lipase (HL) was measured using an enzymatic determination of the released free fatty acids [13]. Substrates were prepared for LPL in pH 8.2 and for HL in pH 8.8. Selective assays of LPL and HL were feasible by inactivating HL with sodium dodecyl sulfate and by inactivating LPL with 1 M NaCl, respectively. The concentrations of free fatty acids were measured by an enzymatic kit (NEFA kit-k; Nippon Shoji Kaisha, Ltd., Tokyo, Japan). Plasma cholesterol ester transfer protein (CETP) activity was measured as reported previously [14].

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Materials and Methods

In this study, we bred 2 colonies of WHHL rabbits based on the plasma levels of TG, as described previously: TGH-WHHL and TGL-WHHL rabbits [11]. Rabbits used for this study were male and 4, 7 and 12 months old. Age- and sex-matched Japanese white (JW) rabbits from Kumagai (Saitama, Japan) were used as a normal control. The 3 groups of rabbits were maintained individually in a controlled environment with free access to water and a chow diet (Labo-R-Grower, Nihon Nosan Kogyo, Ltd., Tokyo, Japan). All animal experiments were performed with the approval of the Animal Care Committee of the University of Yamagata and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

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Results

Basal Concentrations of Plasma Lipids and Body Weight

As shown in Table 1, 2 WHHL rabbits exhibited marked hyperlipidemia (12- to 16-fold increase in TC and 3- to 17-fold increase in TG at 6 months) compared with normal JW rabbits. When a comparison was made between TGH-WHHL and TGL-WHHL rabbits, plasma TG levels of TGH-WHHL rabbits were 6-fold (6 months) and 3.8-fold (12 months) higher than those of TGL-WHHL rabbits. In addition, plasma TC levels were also higher in TGH-WHHL than in TGL-WHHL rabbits. There was no difference in body weight between WHHL and normal JW rabbits.

TG Secretion and Catabolic Rates in vivo

To explore the possible mechanism for higher TG levels found in TGH-WHHL rabbits, the Triton WR-1339 experiment was performed. The TGSR was calculated from the linear fitting of a TG time-concentration relationship. The TGSR of TGH-WHHL rabbits was significantly higher than that of TGL-WHHL and JW groups (Fig. 1). However, the TGSR of TGL-WHHL rabbits was similar to that of JW rabbits even though plasma TG levels of TGL-WHHL rabbits were higher than those of JW rabbits, suggesting that different mechanisms may be operative in 2 WHHL rabbits. The FCR was markedly decreased in both TGH-WHHL and TGL-WHHL rabbits compared with JW rabbits.

TG Secretions in Cultured Hepatocytes

TG and TC contents in the conditioned media produced by cultured hepatocytes were increased in a time-dependent manner in all groups (Fig. 2). However, hepatocytes of TGH-WHHL rabbits produced a significantly higher amount of both TG and TC than those in TGL-WHHL rabbits. Hepatic production of TG and TC in TGL-WHHL rabbits showed an increased tendency compared with JW rabbits, but this was not statistically significant.

Activity of LPL, HTGL and CETP

Post-heparin plasma LPL activity of TGH-WHHL rabbits was significantly lower than that of TGL-WHHL rabbits with Hypertriglyceridemia.
rabbits (7.7 ± 1.6 mmol/ml/h in TGH-WHHL vs. 14.3 ± 1.0 mmol/ml/h in TGL-WHHL; p < 0.01; n = 6 for each group), while there was no significant difference in HTGL activity between the 2 WHHL rabbits (data not shown). The levels of plasma CETP activity were higher in TGH-WHHL rabbits than in the other 2 groups (7.8 ± 1.2 nmol/ml/min in TGH-WHHL vs. 4.2 ± 1.3 nmol/ml/min in TGL-WHHL; p < 0.01). There was a linear relationship between CETP activities and plasma levels of TG but not TC in TGH-WHHL rabbits (data not shown).

**Intravenous Glucose Tolerance Test**

Both TGH-WHHL and TGL-WHHL rabbits at fasting state exhibited hyperglyceridemia, but TGH-WHHL rabbits also showed hyperinsulinemia compared with TGL-WHHL and JW rabbits (table 2). The response of PG and insulin to an intravenous glucose load was examined by IVGTT. As shown in figure 3 and table 2, both TGH-WHHL and TGL-WHHL rabbits showed impaired glucose clearance after 120 min, but TGH-WHHL rabbits were more prominent than TGL-WHHL rabbits. The levels of 120 min PG and IRI were apparently higher in TGH-WHHL and TGL-WHHL than in JW rabbits. After analyzing the levels of plasma TG and insulin, we found that correlation coefficients were significant between \( \Sigma \text{PG \& IRI} \) and TG (fig. 4), whereas there was no significant correlation between \( \Sigma \text{PG \& IRI} \) and TG (data not shown).

**Table 2.** Fasting plasma glucose and insulin and sum of IVGTT

<table>
<thead>
<tr>
<th></th>
<th>TGH-WHHL (n = 5)</th>
<th>TGL-WHHL (n = 7)</th>
<th>JW (n = 7)</th>
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<tr>
<td>Fasting PG, mg/dl</td>
<td>170 ± 12^a,b</td>
<td>175 ± 8^c</td>
<td>139 ± 6</td>
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<tr>
<td>Fasting IRI, mIU/ml</td>
<td>11.1 ± 2.3^a,b</td>
<td>5.8 ± 0.7</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>( \Sigma \text{PG, mg/dl} )</td>
<td>3,221 ± 193^a</td>
<td>3,263 ± 114^c</td>
<td>2,745 ± 123</td>
</tr>
<tr>
<td>( \Sigma \text{IRI, mIU/ml} )</td>
<td>152 ± 17.6^a</td>
<td>100 ± 13.4</td>
<td>78 ± 8.9</td>
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</table>

\( \Sigma \text{PG} \) and \( \Sigma \text{IRI} \) are the sum of PG and IRI concentrations at each time point during the IVGTT (fig. 3).

Data are expressed as the mean ± SEM. ^a p < 0.01, TGH vs. TGL; ^b p < 0.01, TGH vs. JW; ^c p < 0.01, TGL vs. JW.

**Histological Examinations of Islets**

We also examined the islet histological changes, and representative micrographs are shown in figure 5. Quantitative analysis of the islet size distribution showed TGH-WHHL rabbits had more hypertrophied islets (>300 \( \mu \text{m} \)) than the other 2 groups.

**Discussion**

In the present study, using 2 kinds of WHHL rabbits with different levels of plasma TG, along with JW wild-type rabbits, we investigated the possible mechanisms responsible for hypertriglyceridemia in TGH-WHHL rabbits. We found that hypertriglyceridemia in TGH-WHHL rabbits was essentially caused by enhanced hepatic production and reduced catabolism of TG-rich lipoproteins. This notion is supported by both the Triton WR-1339 injection study and measurements of TG secretion from isolated hepatocytes: TGH-WHHL rabbits had a higher capability of TG production compared with TGL-WHHL or JW groups. In combined hyperlipidemia, increased
production of apoB-100 is a common underlying metabolic characteristic [19]. The microsomal TG transfer protein (MTP) plays an obligatory role in the production of apoB-containing lipoproteins [20]. In the present study, there were significant increases in both TG and TC levels in TGH-WHHL rabbits compared with TGL-WHHL or JW rabbits; however, the MTP mRNA expression in both the liver and small intestine was not significantly increased by Northern blotting (data not shown), suggesting that there is an alternative pathway independent of MTP in the pathogenesis of hypertriglyceridemia of TGH-WHHL rabbits.

In the present study, TGH-WHHL rabbits also show a higher activity of CETP compared with TGL-WHHL and JW rabbits. However, it is not known whether high CETP directly causes hypertriglyceridemia in TGH-WHHL rabbits.

TG catabolism is mediated by several factors, including LPL-mediated hydrolysis of TG in the peripheral tissue, hepatic uptake at the binding site which recognizes apoE on the surface of parenchymal cells, LDL receptor-mediated catabolism of very low density lipoprotein, and

**Fig. 3.** IVGTT was performed as described in Materials and Methods. Values are expressed as the means ± SEM. n = 5 for TGH-WHHL, n = 7 for TGL-WHHL, and n = 7 for JW rabbits.

**Fig. 4.** Correlation between fasting levels of TG and ΣIRI.
extrahepatic cellular uptake by very-low-density lipoprotein receptors. In the current study, TGH-WHHL rabbits had lower LPL activities than TGL-WHHL rabbits, which is consistent with the suppressed level of the fractional catabolic rate of plasma TG. Therefore, the combined effects of decreased LPL activity and enhanced CETP activities in TGH-WHHL rabbits favor the pathogenesis of hypertriglyceridemia.

Hypertriglyceridemia is a hallmark for metabolic syndrome in humans and is frequently associated with insulin resistance [4]. In the current study, we also demonstrated that TGH-WHHL rabbits had impaired glucose metabolism and insulin resistance. TGH-WHHL rabbits showed an increased number of hypertrophic islets in the pancreas accompanied by hyperinsulinemia. Although it is not completely clear how hypertriglyceridemia affects insulin resistance, the current study indicates that TGH-WHHL rabbits may serve as a model for the study of combined familial hyperlipidemia and metabolic syndrome.

![Fig. 5. Histological analysis of the pancreas. Hematoxylin-eosin (HE) and immunohistochemical staining for insulin (IHC) of islets. ×200.](image)

### Table 3. The distribution of pancreas islets in various sizes

<table>
<thead>
<tr>
<th></th>
<th>250 μm</th>
<th>300 μm</th>
<th>350 μm</th>
<th>&gt;400 μm</th>
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<tr>
<td>TGH (n = 3)</td>
<td>147 ± 31</td>
<td>112 ± 19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54 ± 8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>27 ± 4&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TGL (n = 3)</td>
<td>89 ± 19</td>
<td>54 ± 20</td>
<td>19 ± 6</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>JW (n = 3)</td>
<td>71 ± 14</td>
<td>36 ± 6</td>
<td>10 ± 3</td>
<td>3 ± 0.6</td>
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<sup>a</sup> p < 0.01, TGH vs. TGL; <sup>b</sup> p < 0.01, TGH vs. JW.
In summary, our results have shown that causes of hypertriglyceridemia in TGH-WHHL rabbits are multifactorial: increased synthesis and decreased catabolism of TG-rich lipoproteins along with low LPL and high CETP activities. Our study also suggests that hypertriglyceridemia is closely associated with insulin resistance.

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