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Enhanced aortic atherosclerosis in transgenic Watanabe heritable hyperlipidemic rabbits expressing lipoprotein lipase

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Abstract

Objective: This study was designed to address the effects of increased lipoprotein lipase (LPL) activity on atherosclerosis in the setting of LDL receptor deficiency.
Methods: We generated transgenic (Tg) Watanabe heritable hyperlipidemic (WHHL) rabbits overexpressing human LPL and compared their plasma lipids and aortic atherosclerosis with non-Tg WHHL rabbits.
Results: Increased expression of LPL significantly ameliorated hypertriglyceridemia and hypercholesterolemia in Tg WHHL rabbits [64% reduction in total cholesterol (TC) and 91% reduction in triglycerides (TG) vs. non-Tg]. In spite of this beneficial effect of LPL, Tg WHHL rabbits had two-fold greater aortic atherosclerosis than non-Tg WHHL rabbits. Analysis of plasma lipoprotein profiles revealed that increased LPL activity in Tg WHHL rabbits resulted in the dramatic reduction of large TG-rich lipoproteins (VLDL, d<1.006 g/ml and IDL, d=1.006–1.02) but concomitant increases in LDL fractions, especially those of small and dense LDL particles (d=1.04–1.06, 2.6-fold over non-Tg). Using apoB-containing lipoproteins, we found that small-sized LDL from Tg WHHL rabbits contained more oxidizable substrate and exhibited higher affinity to biglycan than large TG-rich LDL of non-Tg WHHL rabbits.
Conclusions: We conclude that in the absence of LDL receptor function, increased LPL activity accelerates the catabolism of large TG-rich VLDL (possibly via the LRP pathway) and subsequently improves hyperlipidemia. However, LPL may also enhance the generation and accumulation of small dense LDLs, which are more atherogenic.

Keywords: Lipoprotein lipase; WHHL rabbits; Transgenic rabbits; Hypercholesterolemia; Hypertriglyceridemia; Atherosclerosis

1. Introduction

Lipoprotein lipase (LPL) is a rate-limiting enzyme for the hydrolysis of triglyceride-rich lipoproteins and plays crucial roles in the remnant lipoprotein and HDL metabolism [1–3]. LPL is mainly produced by adipose tissue and muscle and is transported to the surface of the capillary endothelium, where LPL is bound to heparan sulphate proteoglycan and exerts its functions [4].

Accumulating evidence has revealed that LPL is essentially an antiatherogenic enzyme [5,6]. This notion has been supported by observations in humans who are deficient in LPL or have LPL gene mutations [7,8]. These patients have premature atherosclerosis. Furthermore, the long-term administration of LPL-activator (NO-1886 compound) in cholesterol-fed rats and rabbits led to the inhibition of

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atherosclerosis [9,10]. In addition, overexpression of the LPL transgene in LDL receptor- and apoE-deficient mice [5,6] or transgenic (Tg) rabbits [11] significantly suppressed diet-induced atherosclerosis. The antiatherogenic effect of LPL may be exerted via two mechanisms: (i) the acceleration of the hepatic removal of remnant lipoproteins and subsequently reduction of plasma lipids and (ii) the elevation of plasma HDL levels [12]. Whether LPL itself is antiatherogenic in the absence of a lipid-lowering effect, however, is still unclear.

Another body of evidence suggests that LPL may be potentially proatherogenic [13,14]. For example, LPL secreted from macrophages in the arterial wall is generally considered to be proatherogenic because inbred mice with higher expression of macrophage LPL have more diet-induced atherosclerosis [15]. Transplantation of macrophages deficient for the LPL gene leads to reduced atherosclerosis in C57BL/6J or LDL receptor-knockout mice [16,17]. Macrophage-specific expression of LPL increases diet-induced atherosclerosis in both Tg mice [18] and rabbits [19]. In addition, LPL may increase monocyte adhesion to endothelial cells [20,21], enhance the retention of LDL particles by the subendothelial extracellular matrix and an interaction with apoB [22–24], and mediate the formation of foam cells [25], although these mechanisms have not been fully verified in vivo.

The functional roles of LPL in LDL metabolism have not been defined, and also it is not known whether increased LPL activity is beneficial for the treatment of familial hypercholesterolemia (FH), in which LDLs rather than remnant lipoproteins are markedly elevated. One study of 15 homozygous FH patients revealed that plasma LPL mass is positively associated with calcific coronary atherosclerosis [26]. Those authors proposed an interesting hypothesis that increased LPL may be a significant risk factor for cardiovascular disease.

To test the hypothesis that LPL plays an important role in LDL metabolism and to examine its effect on FH, we characterized Tg Watanabe heritable hyperlipidemic (WHHL) rabbits, which express high levels of LPL. We found that overexpression of LPL in Tg WHHL rabbits led to a dramatic reduction of plasma lipid levels but greater aortic atherosclerosis compared to that in non-Tg WHHL rabbits. Analysis of the atherogenic potential of lipoproteins revealed that increased small and dense LDL particles may be responsible for the increased susceptibility to atherosclerosis in Tg WHHL rabbits.

2. Methods

2.1. Animals

Tg WHHL rabbits were generated by cross-breeding with LPL Tg rabbits as described previously [27]. In the current study, 11 Tg (5 males and 6 females) and 25 non-Tg (11 males and 14 females) littermate homozygous WHHL rabbits were used. The rabbits were given water and a standard chow diet (REQ, Oriental Yeast, Tokyo, Japan) ad libitum. All animal experiments were performed with the approval of the Animal Research Committee of the University of Tsukuba and with the NIH guidelines.

2.2. Plasma lipid and lipoprotein analyses

Blood was collected from rabbits at 3, 6 and 11 months of age after 16 h of food deprivation. Plasma total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), and free

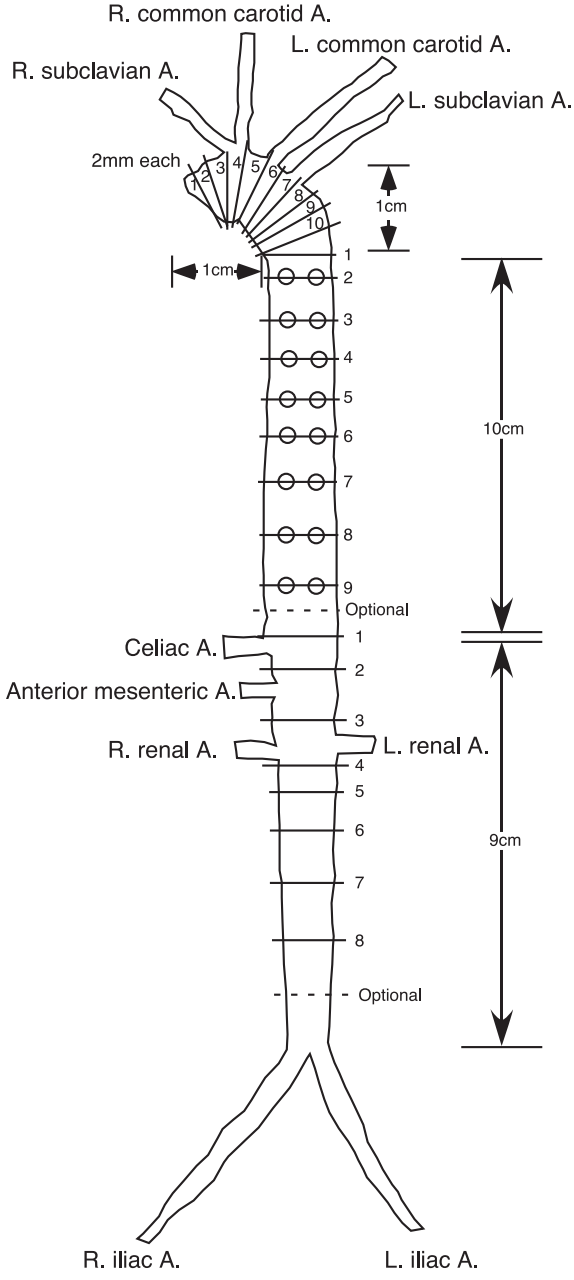

Fig. 1. Schematic illustration of the whole arterial tree of rabbits and the lesion sites for section analysis. The whole aorta is divided into three parts: aortic arch (10 sections), thoracic aorta (9 sections), and abdominal aorta (8 sections).

fatty acids (FFA) were measured using Wako analysis kits [27]. The enzymatic activity of LPL in the post-heparin plasma was determined using ^{14}C-labeled triolein emulsion substrate as described before [11]. For the determination of the apolipoprotein distribution in Tg WHHL rabbit lipoproteins, plasma lipoproteins were isolated by sequential ultracentrifugation [28]. These lipoprotein fractions were subjected to agarose gel electrophoresis and stained with Fat red 7B or transferred to a nitrocellulose membrane for immunoblotting [29]. In addition, isolated lipoprotein density fractions were examined by negative stain electron microscopy as described [30].

2.3. Quantification of atherosclerotic lesions

Rabbits were sacrificed at 11 months by venous injection of an overdose of sodium pentobarbital solution. The whole aortas were stained with Sudan IV for the evaluation of the gross size of atherosclerotic lesions [31]. The en face sudanophilic area (surface involvement) relative to the surface area of each aorta segment was measured using a MacScope image analysis system. For the microscopic evaluation of the lesion size and quality, the entire aortic tree was cut serially into sections as shown in Fig. 1 and the intimal and medial lesion sizes were quantitatively measured using the MacScope system and expressed as microscopic lesion areas [30]. In addition, the lesion types (early stage lesions consisting of types I and II, advanced lesions consisting of types III to V) were evaluated based on the criteria of American Heart Association. To study cellular components, lipoprotein and LPL deposits in the lesions, we performed immunohistochemical staining using the following antibodies (Abs) against macrophages (Mϕ) (RAM11), smooth muscle α-actin (HHF35), apoB, oxidized LDL (FOH1a/DLH3) [32], and LPL (5D2). The relative distribution of smooth muscle cells (SMC) and Mϕ in the lesions was determined using the MacScope system [33].

2.4. Evaluation of apoB-containing lipoprotein in vitro

To evaluate the atherogenecity of apoB-containing particles, we isolated four density fractions from both Tg and non-Tg WHHL rabbits. They were lipoproteins with d<1.006 g/ml (VLDL), d=1.006–1.02 g/ml (IDL), d=1.02–1.04 g/ml (containing both IDL and large LDL), and d=1.04–1.06 g/ml (HDL_1 and small and dense LDL). In this study, we compared the susceptibility of these lipoproteins to copper-induced oxidation and their affinity to the extracellular matrix biglycan as described [30]. Absorbance values were used to make an indirect evaluation of the oxidation degree and binding affinity of these lipoproteins.

2.5. Statistical analysis

All values were expressed as mean±S.E. and statistical significance was determined using Student's, Welch's t-test or Mann–Whitney's U-test for nonparametric analysis. In all cases, statistical significance was set at p<0.05.

3. Results

3.1. Effect of increased LPL on plasma lipid levels

The LPL activity of Tg WHHL rabbits in post-heparin plasma was 3-fold (in males) and 1.8-fold (in females) higher than that of sex-matched non-Tg WHHL littermates (p<0.01). The increased LPL activity in males led to a constant and significant reduction of plasma TC and TG levels at 3, 6, and 11 months compared to non-Tg WHHL

Table 1
Plasma lipids in Tg and non-Tg WHHL rabbits

		TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	FFA (mEq/l)
Male					
Non-Tg WHHL (n=11)	3 months	915±50	205±39	10.3±1.7	0.50±0.03
Tg WHHL (n=5)		308±30**	11±2**	7.5±1.2	0.37±0.02*
	6 months	697±59	169±24	8.7±1.7	0.53±0.04
		258±25**	12±2**	12.2±1.1	0.51±0.05
	11 months	458±42	227±28	12.8±1.5	1.00±0.11
		164±33**	31±6**	17.4±3.1	0.53±0.14*
Female					
Non-Tg WHHL (n=14)	3 months	592±38	220±21	6.5±1.1	0.46±0.05
Tg WHHL (n=6)		720±80	85±17**	7.3±1.3	0.54±0.05
	6 months	610±47	204±20	12.9±2.1	0.50±0.04
		700±143	69±15**	18.9±6.9	0.53±0.06
	11 months	452±36	290±55	9.9±1.9	0.64±0.13
		441±46	72±19*	8.8±0.4	0.69±0.27

Results are expressed as mean±S.E. Statistical significance was determined by Student's or Welch's t-test. Rabbits were maintained chow diet and then fasted for 16 h before analysis.

* p<0.05 vs. non-Tg WHHL rabbits.

** p<0.01 vs. non-Tg WHHL rabbits.

rabbits (Table 1). In addition, FFA levels in male but not female Tg WHHL rabbits were also reduced at 3 and 11 months. In female Tg WHHL rabbits, however, TC levels in plasma were not significantly changed compared to those in non-Tg WHHL rabbits, although hypertriglyceridemia was mildly improved. There were no significant differences in HDL-C levels between the two groups.

3.2. Quantification of atherosclerotic lesions

In male Tg WHHL rabbits, which had lower levels of TC [164±33 mg/dl, at 11 months; normal male Japanese White (JW) rabbits: 30–50 mg/dl in our facility] and TG (31±6 mg/dl; close to normal JW rabbits: 30–60 mg/dl), aortic lesions were surprisingly and significantly greater than those in non-Tg WHHL rabbits: 1.5-fold increased in the aortic arch ($p<0.05$), 2.4-fold increased in the thoracic aorta ($p<0.01$), and 1.3-fold increased in the abdominal aorta ($p=0.3$, not statistically significant; Fig. 2A and B). In female Tg WHHL rabbits, the mean lesion area in the abdominal aorta was increased by 2-fold compared non-Tg WHHL rabbits ($p<0.05$). It should be noted that the lesions in aortic arch and thoracic aorta occupied almost the whole areas (saturated) in female Tg and non-Tg rabbits.

Next, we examined the lesion histological characteristics in male rabbits under a light microscope. For this purpose, we selected the aortic arch and thoracic aorta because the lesions in these areas were consistently present in all Tg and non-Tg WHHL rabbits. In the thoracic aorta, atherosclerotic lesions of Tg WHHL rabbits were 2.9-fold larger than those of non-Tg WHHL rabbits (Fig. 3A), whereas the lesion size in the aortic arch was not significantly different (data not shown). We also compared the lesion types in an attempt to determine which types of lesions were increased in Tg WHHL rabbits. As shown in Fig. 3B, the distribution of types of lesions in Tg WHHL rabbits was similar to that in non-Tg WHHL rabbits (35% early stage lesions and 65%

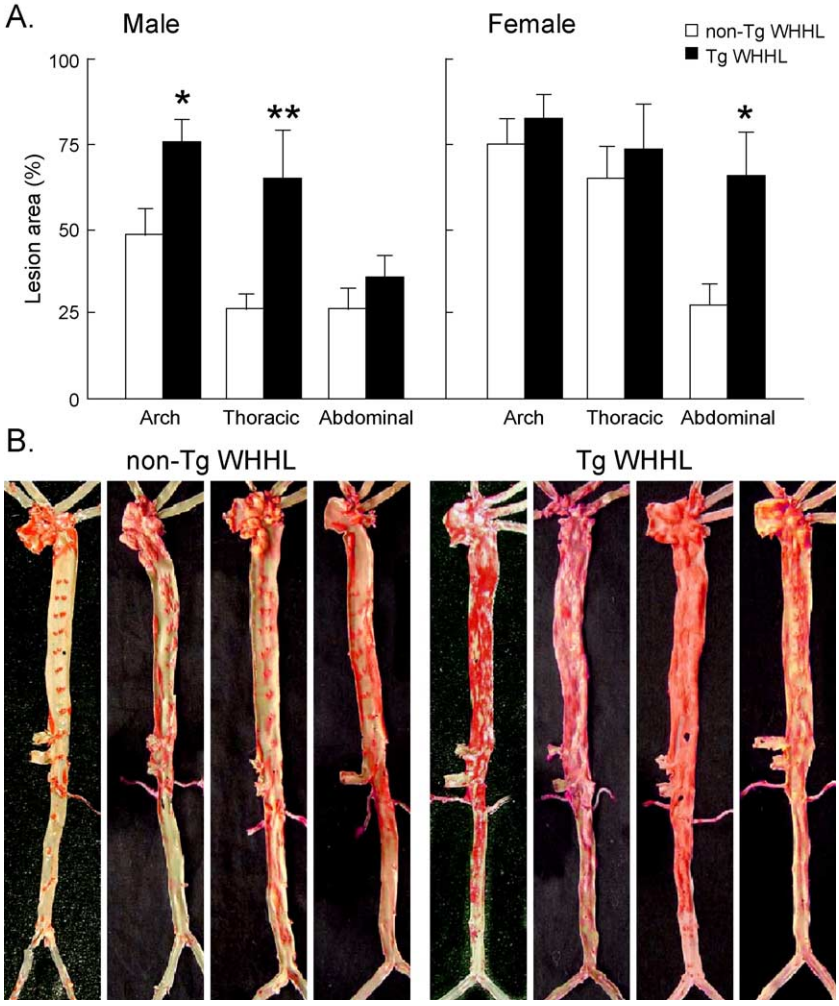

Fig. 2. Quantitative analysis of aortic atherosclerosis. The lesion area (Sudanophilic area) in each aortic segment was determined as described in Methods and is shown in the upper panel (A). Data are expressed as mean±S.E. $n=5$ and 10 for Tg and non-Tg WHHL rabbits, respectively. *$p<0.05$ and **$p<0.01$ vs. non-Tg. Representative photographs of the pinned-out aortic trees (male rabbits) stained with Sudan IV are shown in the lower panel (B).

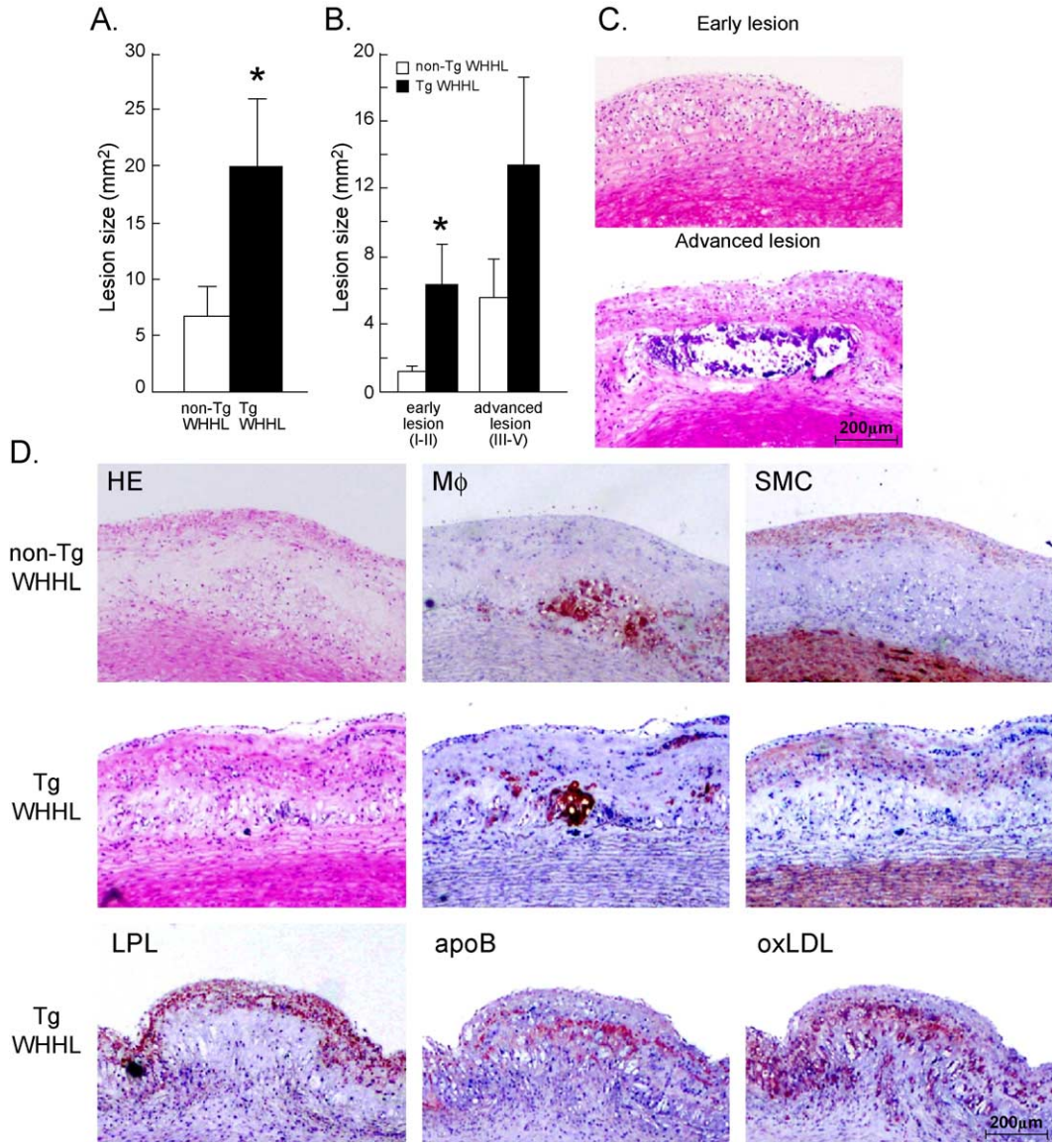

Fig. 3. Microscopic analysis of lesion area. Cross-sections of the thoracic aorta from each animal were stained with EVG and the intimal lesion area was quantitated using an image analysis system (A). The distribution of each lesion (early vs. advanced) was quantitated using HE-stained specimens (B). Representative lesion micrographs stained with HE are shown (C). Data are expressed as mean±S.E. n=5 and 9 for Tg and non-Tg rabbits, respectively. * $p<0.05$ vs. non-Tg. Representative micrographs immunostained with SMC, Mϕ, LPL, apoB and oxLDL Abs are shown in D. Original magnification is 50× for C and D.

advanced lesions), but there was clearly a significant increase of early stage lesions in Tg WHHL rabbits. Representative early and advanced lesions from Tg WHHL rabbits are shown in Fig. 3C.

Finally, we analyzed the cellular components (Mϕ vs. SMC) of the lesions by immunohistochemical staining. Cellular components (stained with Abs against either Mϕ or SMC) occupied about 20–25% of the lesion area in both Tg and non-Tg WHHL rabbit lesions. There was a tendency for the SMC population in atherosclerotic lesions of the thoracic aorta in Tg WHHL rabbits to be increased compared to that in non-Tg WHHL rabbits, although the increase was not significant, whereas Mϕ were not different between the two groups (data not shown). In the lesions from Tg WHHL rabbits, LPL immunoreactive proteins were detected in SMC-rich areas beneath which apoB and oxidized LDL were frequently observed (Fig. 3D, lower panel).

3.3. Analyses of plasma lipoprotein profiles

Since Tg WHHL rabbits had greater atherosclerotic lesions, we hypothesized that LPL may mediate lipoprotein metabolism and altered lipoprotein profiles may be responsible for the enhancement of atherosclerosis. Therefore, we characterized the lipoprotein profiles using sequential density

gradient ultracentrifugation. As expected, overexpression of LPL in Tg WHHL rabbits led to a dramatic reduction of VLDL (86%) and IDL (88%) cholesterol contents compared to those in non-Tg WHHL rabbits. However, Tg WHHL rabbits had an increased small LDL cholesterol levels: a 1.3-fold increase in fractions with d=1.04–1.06 g/ml and a 2.1-fold increase in fractions with d=1.06–1.08 g/ml over non-Tg levels (Fig. 4, upper panel). The increase of small LDL content became more obvious when the values were plotted as a relative distribution expressed as percentage (Fig. 4, middle panel). Clearly, there was a prominent increase of small LDLs in Tg WHHL rabbits and these LDLs carried 21% of TC in Tg compared to 8% in non-Tg WHHL rabbits. TG contents were reduced in all seven fractions of Tg WHHL rabbits (Fig. 4, bottom panel).

The distribution of apolipoproteins among the various density fractions was examined by Western blotting of lipoproteins that had been resolved by agarose gel electrophoresis (Fig. 5A and B). In Tg WHHL rabbits, decreased VLDL and IDL (d<1.006 and d=1.006–1.02 g/ml) were associated with reduced contents of apoE and apoB. Of note, the increased small LDL fractions (d=1.06–1.08 g/ml) in Tg WHHL rabbits were enriched in apoB and apoE. We also examined the ratio of apoB100 and apoB48 in VLDL fractions from both fasting and postprandial animals as shown in Fig. 5B. VLDL from Tg WHHL rabbits had lower apoB100 in both fasting and postprandial states, and the apoB48 levels of these VLDL fractions were remarkably lower than those from non-Tg WHHL rabbits. In female Tg WHHL rabbits, there was a reduction of VLDL, although it was not as prominent as that in males (data not shown). They had a significant (2.5-fold) increase of small LDL (d=1.04–1.06 g/ml) content compared to non-Tg WHHL rabbits, although their TC levels were similar.

We next examined apoB-containing lipoproteins by transmission electron microscopy. Representative micrographs of five density fractions of apoB-containing particles are shown in Fig. 6A and the average diameters and distribution of the lipoproteins are illustrated in Fig. 6B. The mean size of lipoproteins with d<1.006 and d=1.006–1.02 g/ml in Tg WHHL rabbits was larger than that in non-Tg WHHL rabbits, presumably due to the decrease of small VLDL particles (<33 nm in d<1.006, <20 nm in d=1.006–1.02 g/ml) and relative increase of large VLDL particles (>66 nm in d<1.006, >30 nm in d=1.006–1.02 g/ml) in Tg WHHL rabbits. In fractions with d=1.04–1.06 g/ml, there were two populations of particles, large-sized particles (small LDL, >15 nm) and small-sized particles (HDL_1, <15 nm) in non-Tg WHHL rabbits. In contrast, in Tg WHHL rabbits, the large-sized particles predominated in the whole population. Small and denser LDL particles in the fractions with d=1.06–1.08 g/ml were reduced in size compared to those of non-Tg WHHL rabbits, possibly due to enhanced lipolysis process in Tg WHHL rabbits. In the fractions with d=1.04–1.08, there were some rod-shaped particles (Fig. 6A) which may presumably represent LDL particles in the process of hydrolysis.

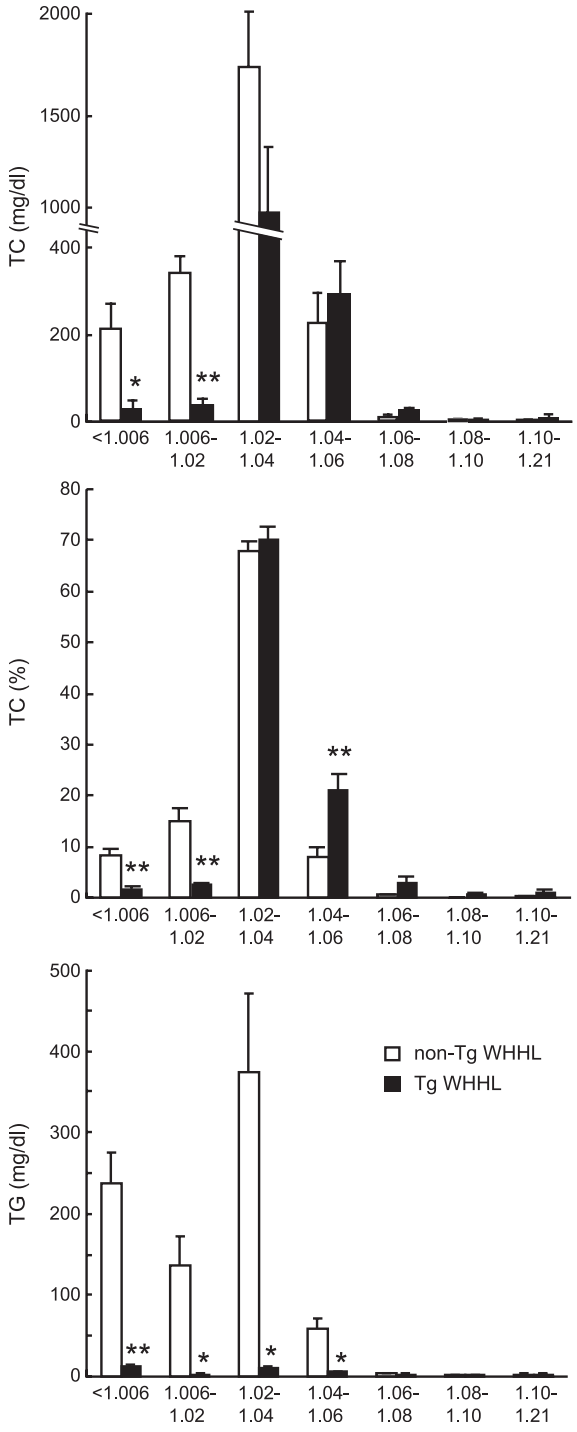

Fig. 4. Quantitation of TC and TG contents in plasma lipoproteins. Density gradient fractions were isolated from male Tg WHHL rabbits at the age of 8 months by ultracentrifugation and lipid contents were measured as described. Top panel shows TC levels. To permit a comparison between Tg and non-Tg WHHL rabbits, the relative distribution of cholesterol among the various fractions was plotted and expressed as percentage (middle panel). Lower panel indicates TG levels. The combined recovery of cholesterol from each animal averaged ~80% of total plasma levels. Data are expressed as mean±S.E. *p<0.05 and **p<0.01 vs. non-Tg.

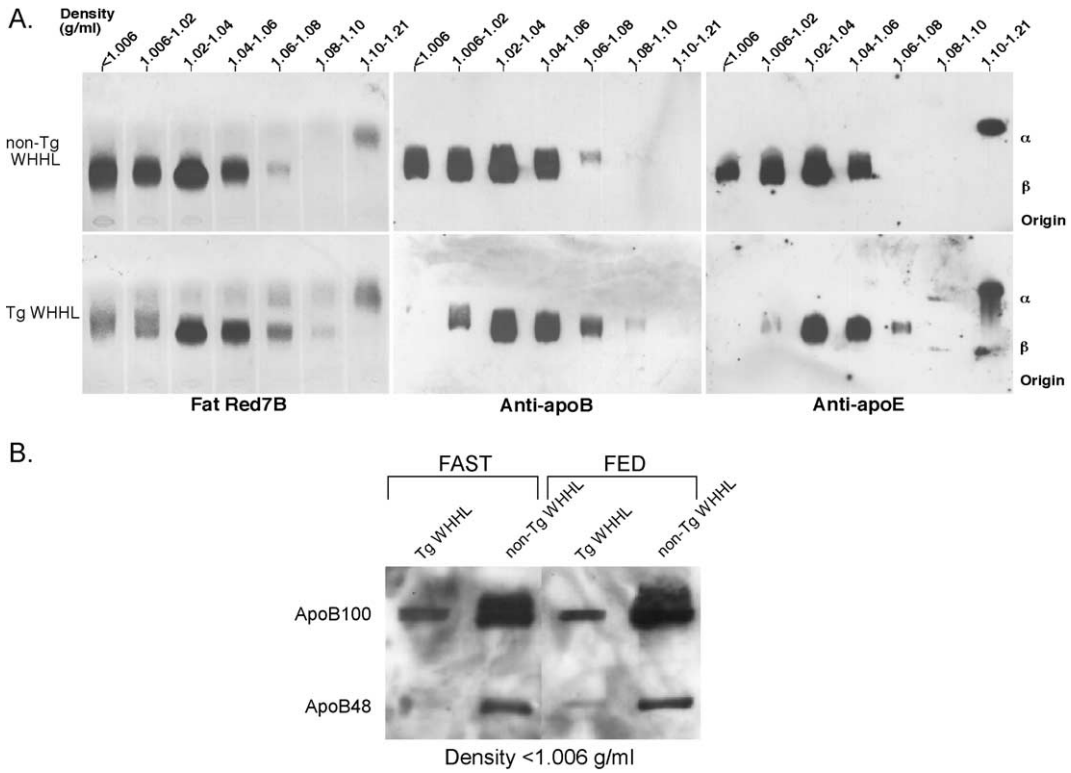

Fig. 5. Plasma lipoprotein analysis. Lipoproteins from male rabbits were resolved by electrophoresis in a 1% agarose gel and visualized with Fat Red 7B staining and apolipoproteins were detected by immnoblotting with specific antibodies against apoB and apoE (A). For comparison of apoB levels in VLDL (d<1.006 g/ml), the plasma was collected from fasting and fed rabbits and density fractions were isolated by ultracentrifugation, and resolved by 3.5% SDS-PAGE and stained with Coomassie brilliant blue (B).

3.4. Analyses of atherogenic properties of apoB-containing lipoproteins

Since Tg WHHL rabbits had more extensive lesions than non-Tg WHHL rabbits, regardless of having significantly lower levels of TC and TG, we next sought to gain insight into the mechanism of the enhancement of atherosclerosis in Tg rabbits. The difference in the lipoprotein profiles between the two groups of rabbits led us to hypothesize that LDL particles (especially small LDL) may be more atherogenic than particles of remnant lipoproteins. To explore this possibility, we isolated four apoB-containing lipoprotein fractions (d<1.006, d=1.006–1.02, 1.02–1.04, and 1.04–1.06 g/ml) as shown in Figs. 4, 5 and 6, and compared their susceptibility to copper-induced oxidization in vitro. In non-Tg WHHL rabbits, VLDL showed the highest degree of oxidization (as expressed by the production of conjugated dienes) among the four fractions, whereas in Tg WHHL rabbits, LDL fractions (d=1.02–1.06 g/ml) were more sensitive to copper-induced oxidation than large-sized VLDLs (lag time: 71.7 and 44.7 min in d=1.02–1.04 and 1.04–1.06 g/ml vs. not detectable and 78.2 in d<1.006 and 1.006–1.02 g/ml). When the comparison was made between Tg and non-Tg rabbits, the differences of oxidization degree between VLDL and LDL fractions were remarkable whereas the changes in IDLs (d=1.006–1.02 g/ml) were not obvious (Fig. 7A).

Furthermore, we compared the ability of the apoB-containing lipoproteins to bind to biglycan, a major component of proteoglycans, a process that are supposedly involved in atherogenic lipoprotein retention in the lesions [34]. VLDL fractions (at the same amount of proteins) isolated from Tg WHHL rabbits had the same binding ability as those from non-Tg WHHL rabbits; however, the other three fractions from Tg WHHL rabbits showed higher affinity to biglycan than those from non-Tg WHHL rabbits (Fig. 7B).

4. Discussion

In this study, we investigated the effects of increased LPL activity on the LDL metabolism in WHHL rabbits, an animal model of human FH. WHHL rabbits have high levels of LDL cholesterol and spontaneous atherosclerosis caused by the defect of LDL receptors. This study also allowed us to examine whether genetic manipulation of LPL can be used to treat atherosclerosis of FH patients. As expected, overexpression of LPL efficiently ameliorated hypertriglyceridemia of both male and female rabbits, and in males, also hypercholesterolemia. This lipid-lowering effect of LPL was caused by the fact that overexpression of LPL decreases VLDL and IDL levels, possibly via the LDL receptor-related protein (LRP) pathway. A number of

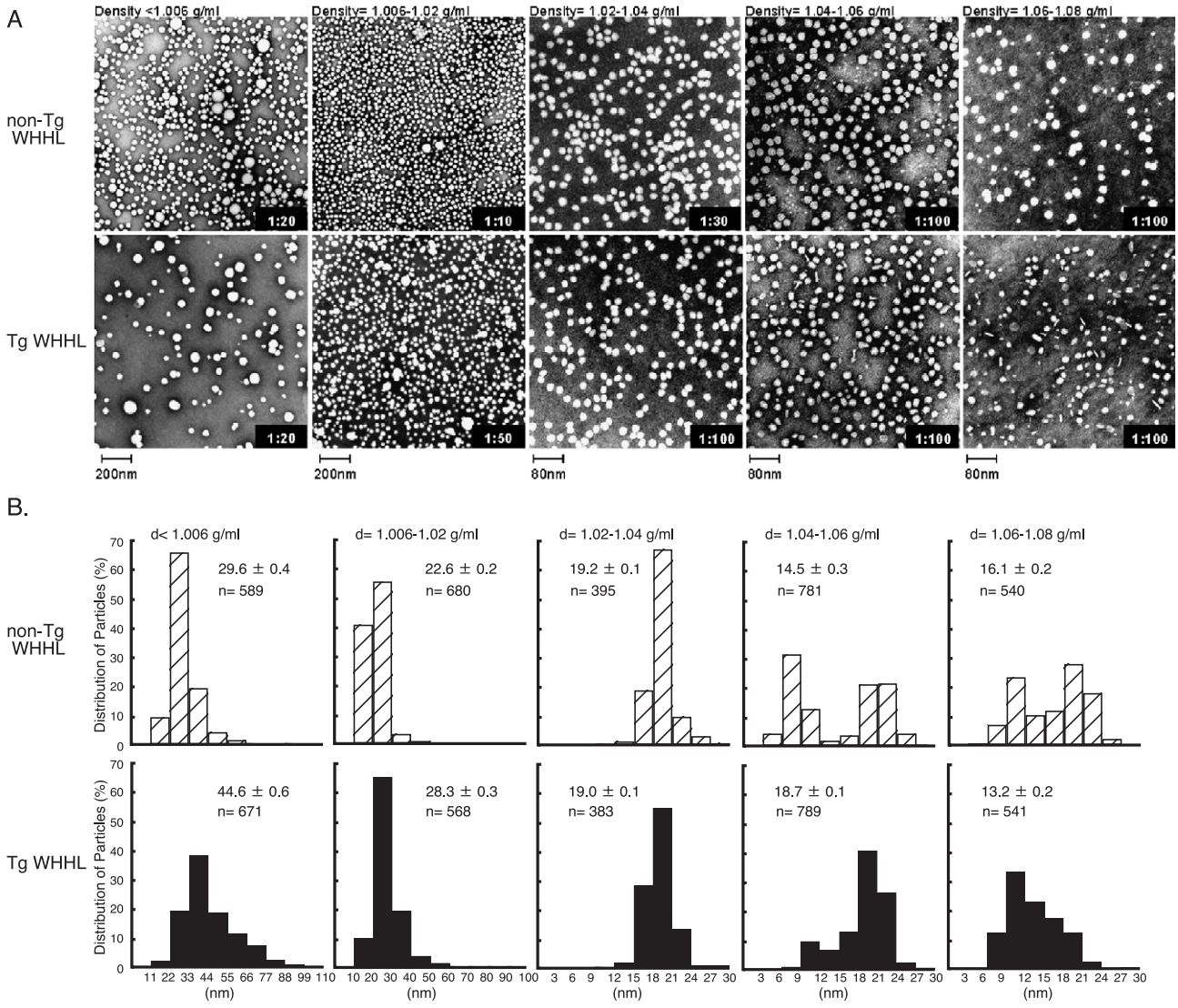

Fig. 6. Electron microscopic analysis of lipoproteins. Lipoproteins from male rabbits were examined by negative stain electron microscopy and measured using an image analysis system as described in Methods. Representative micrographs of five fractions (d<1.006–1.08 g/ml) are shown in A. Lipoproteins were diluted as shown at the bottom of the picture. The distribution of the lipoproteins with various sizes is illustrated in B. The average particle diameter±S.E. and the total particles counted are listed below the name of each lipoprotein class.

studies have shown that LPL participates in the clearance of VLDL and remnants through LRP [22]. The reason for different effects of LPL on cholesterol levels between male and female WHHL rabbits is unknown but may be due to the LPL expression levels since male Tg had higher LPL activity than female Tg rabbits.

In spite of having lower plasma lipids, Tg WHHL rabbits had greater aortic atherosclerosis than non-Tg WHHL rabbits. This finding was initially surprising since in male Tg WHHL rabbits, plasma levels of TC and TG were clearly lower than those in their littermate non-Tg WHHL rabbits (64% and 91% reduction, respectively) and also below the atherogenic levels observed in rabbits and considered to apply to humans. Analysis of lipoprotein density fractions revealed that despite having lower levels of VLDL and IDL, Tg WHHL rabbits had higher levels of small LDL than non-Tg WHHL rabbits, suggesting that elevated small LDL levels in Tg rabbits are responsible for increased atherosclerosis in Tg WHHL rabbits. These small LDLs were denser and contained less TG contents compared to LDLs from non-Tg WHHL rabbits, suggesting that increased LPL may be directly involved in the modification of LDL particles. Several mechanisms may be responsible for the increased small LDL levels observed in Tg WHHL rabbits. First, increased LPL levels in Tg WHHL rabbits may accelerate the process of production and/or conversion of IDL to LDL particles. Secondly, increased small LDL levels in plasma may be caused by the LDL receptor deficiency in WHHL rabbits, since these particles cannot be catabolized through LRP, or, small LDL particles cannot compete with apoE-rich particles (VLDL and IDL) for

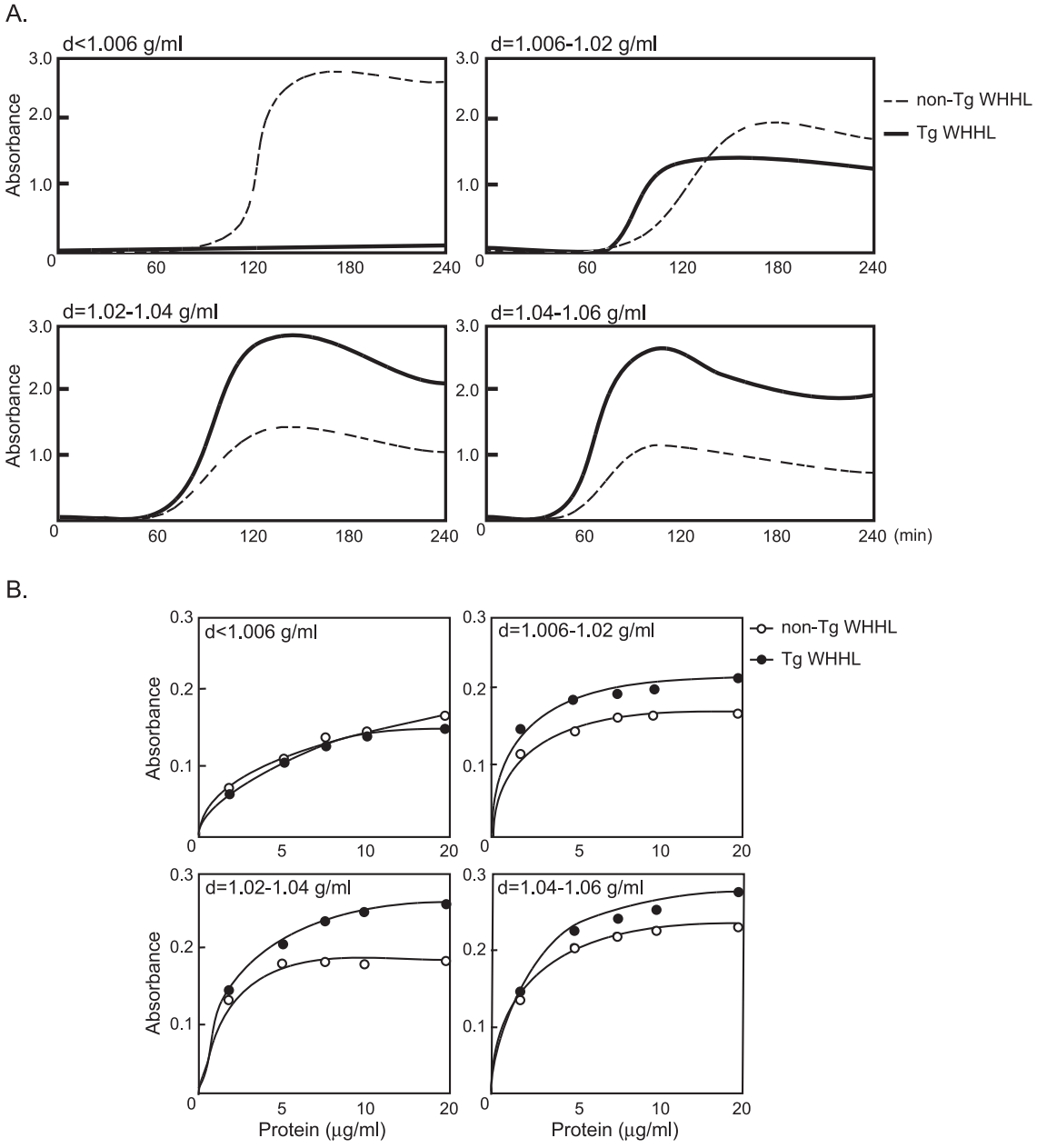

Fig. 7. Kinetics of apoB-containing lipoprotein oxidation and binding to biglycan. Four apoB-containing lipoprotein fractions were isolated from either Tg or non-Tg male WHHL rabbits as described in Methods. Equal amount of lipoproteins from each group was dissolved in PBS (50 μg/ml of protein conc.) [30]. Representative results (n=3) are shown in A. Binding affinity to biglycan was measured and representative results (n=3) are shown in B. Each well was loaded with an equal amount of the indicated lipoprotein fraction and incubated for 1 h.

the limited number of LRPs in the absence of LDL receptor. This assumption is supported by our previous study using cholesterol-fed LPL rabbits [30] in which there was also an accumulation of small LDL, presumably due to the down-regulation of LDL receptors induced by feeding high-cholesterol diets [35,36]. Dugi et al. [26] demonstrated that increased LPL mass and activity are positively correlated with the extent of calcific atherosclerosis in homozygous FH patients, although they could not directly differentiate whether increased LPL is a cause or consequence of the atherosclerosis. Our current study using Tg WHHL rabbits not only support Dugi's study but also provide a direct mechanism for increased atherosclerosis in FH patients. First, elevated small LDLs are more atherogenic regardless of the lower plasma lipids (either lower TG and TC in males or lower TG alone in females), because these particles are easily trapped in the subendothelial matrix [37]. Secondly, prolonged retention time of these particles may increase their susceptibility to chemical modifications, such as oxidation, and thus accelerate the formation of foam cells of both Mϕ and SMC [25,38].

These two possibilities were substantiated by our in vitro studies showing that small LDL particles contain more oxidizable substrates and have higher affinity for the extracellular matrix biglycan. It should be pointed out that LPL itself in the arterial wall can directly participate in these processes as well [39]. Tg WHHL rabbits also expressed the LPL transgene in the peritoneal Mϕ (data not shown). Although we cannot exclude the possibility that Mϕ-derived LPL may be partly responsible for increased atherosclerosis in Tg WHHL rabbits, our previous studies using Mϕ-specific LPL Tg rabbits showed that the induction of lesions by Mϕ-LPL expression was less prominent [19] than that by systemic LPL expression in Tg rabbits that had high levels of small LDLs [30]. The atherogenicity of Mϕ-derived LPL cannot become operative unless animals develop hyperlipidemia induced by an atherogenic diet [17,18]. In the current study, male Tg WHHL rabbits had nearly normal levels of cholesterol in the plasma, which is also different from cholesterol-fed rabbit study [30]. Furthermore, the lesions of Tg WHHL rabbits contained more SMC than Mϕ. Nevertheless, our study provided an explanation for why administration of compound NO-1886 LPL activator has no effect on the inhibition of atherosclerosis in WHHL rabbits [40]. Our finding in WHHL rabbits differs from the report by Shimada et al. [5], who found that in LDL receptor-knockout (LDLr-KO) mice, overexpression of LPL significantly (18-fold reduction over control) suppressed diet-induced atherosclerosis. The discrepancy in terms of LPL antiatherogenic effect in LDLr-KO mice and proatherogenic effect in WHHL rabbits may be attributed to the intrinsic species differences in lipoprotein metabolism and LDL receptor functions. Although both kinds of animals are deficient in LDL receptors, hypercholesterolemia on a chow diet is apparently different (e.g., cholesterol levels are 225±27 mg/dl in LDLr-KO mice [41] vs. 915±50 mg/dl in WHHL rabbits at 3 months) and spontaneous atherosclerosis is clearly stronger in WHHL rabbits than that in LDLr-KO mice since the latter usually require a cholesterol-rich diet to produce hyper-remnant-emia. Furthermore, mice do not have cholesterol ester transfer protein (CETP) and their major lipoproteins are HDL (in normal conditions) and apoB48-containing remnant lipoproteins (in diet-induced hypercholesterolemia), whereas rabbits, like humans, have abundant CETP and high levels of LDL particles [42]. Therefore, elevation of LPL activity may have dual or opposing effects in terms of lipoprotein metabolism: beneficial to those with remnant-rich hyperlipidemia (such as postprandial hyperlipidemia) but detrimental to those with LDL hypercholesterolemia (such as FH). In light of such a complex function of LPL, elevation of LPL activity may not be used as a therapeutic modality for the treatment of atherosclerosis in the setting of LDL receptor deficiency, and a beneficial effect of LPL may not be achieved except in the presence of normal LDL receptor function.

4.1. Conclusion and limitations

In conclusion, our results for the first time showed that increased LPL expression in WHHL rabbits resulted in the elevation of small LDL in plasma and the enhancement of atherosclerosis despite having a lipid-lowering effect. Although elevation of LPL activity may be beneficial in some types of hyperlipidemias, especially hypertriglyceridemia, this strategy may compromise the treatment of atherosclerosis in FH patients in whom LDL receptors are abnormal. It remains to be determined whether the current finding is directly relevant to human; however, there is a need to examine FH patients to address this issue in clinical studies in the future.

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