Overexpression of Lipoprotein Lipase in Transgenic Watanabe Heritable Hyperlipidemic Rabbits Improves Hyperlipidemia and Obesity*

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Lipoprotein lipase (LPL) is the rate-limiting enzyme for the hydrolysis of the triglyceride-rich lipoproteins and plays a crucial role in lipoprotein and free fatty acid metabolism. Genetic manipulation of LPL may be beneficial in the treatment of hypertriglyceridemia, but it is unknown whether increased LPL activity may be effective in lowering plasma cholesterol and improving insulin resistance in familial hypercholesterolemic patients. To test the hypothesis that stimulation of LPL expression may be used as an adjunctive therapy for treatment of homozygous familial hypercholesterolemia, we have generated transgenic (Tg) Watanabe heritable hyperlipidemic (WHHL) rabbits that overexpress the human LPL transgene and compared their plasma lipid levels, glucose metabolism, and body fat accumulation with those of non-Tg WHHL rabbits. Overexpression of LPL dramatically ameliorated hypertriglyceridemia in Tg WHHL rabbits. Furthermore, increased LPL activity in male Tg WHHL rabbits also corrected hypercholesterolemia (544 ± 52 in non-Tg versus 227 ± 29 mg/dl in Tg, p < 0.01) and reduced body fat accumulation by 61% (323 ± 27 in non-Tg versus 125 ± 21 g in Tg, p < 0.01), suggesting that LPL plays an important role in mediating plasma cholesterol homeostasis and adipose accumulation. In addition, overexpression of LPL significantly suppressed high fat diet-induced obesity and insulin resistance in Tg WHHL rabbits. These results imply that systemic elevation of LPL expression may be potentially useful for the treatment of hyperlipidemias, obesity, and insulin resistance.

Lipoprotein lipase (LPL)† plays a pivotal role in lipoprotein metabolism by catalyzing the hydrolysis of triglyceride-rich (TG-rich) lipoproteins such as chylomicrons and very low density lipoproteins (VLDL) (1–3). Through the hydrolysis of TG in these particles, LPL converts these lipoproteins to small, dense particles and results in the generation of surface remnants, which give rise to high density lipoproteins (HDL). This process also generates free fatty acids (FFA), which are taken up and used for metabolic energy in peripheral tissues such as muscle or reesterified into TG and stored in adipose tissue. Therefore, LPL is an important mediator for maintaining whole body energy homeostasis and fat accumulation in adipose tissue (4, 5). LPL is mainly produced by mesenchymal cells such as adipose and muscle cells and then transported to the luminal surface of the vascular endothelium, where it is bound to heparan sulfate proteoglycans. In addition, small amounts of LPL are secreted from other tissues such as adrenals, kidneys, pancreatic islet cells, and macrophages (4).

In humans, patients who are homozygous or heterozygous for LPL gene mutations show elevated plasma TG and reduced HDL levels (2), which in some cases are associated with premature atherosclerosis (6–8). These observations have led to the notion that the elevation of LPL activity either by gene therapy (9, 10) or LPL-raising drugs (11) may be beneficial for hyperlipidemias. In support of this, transgenic mice (Tg) and rabbits overexpressing an LPL transgene protected against diet-induced hyperlipemia and atherosclerosis (12–14). Adenovirus-mediated transient expression of LPL in LPL or apo-lipoprotein E knock-out mice corrected hyperlipidemia and improved fat tolerance (9, 10). Similarly, administration of drugs (NO-1886 or fenofibrate) that enhance the action of LPL resulted in lowering plasma TG levels, improved the capacity to handle fat load, and increased HDL-cholesterol (HDL-C) levels in rats and rabbits (11, 15, 16). Patients deficient in LPL have hypertriglyceridemia and high levels of plasma FFA, which may cause insulin resistance and diabetes and can be ameliorated through lowering of TG (17). Despite these beneficial effects on hyperlipidemias, tissue-specific dysfunction of LPL expression has been implicated in the pathogenesis of myo-lipoprotein; HDL-C, high density lipoprotein-cholesterol; HFD, high fat diet; hLPL, human lipoprotein lipase; IVGTT, intravenous glucose tolerance test; IVITT, intravenous insulin tolerance test; LDLr, low density lipoprotein receptor; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; IR, insulin resistance; SEM, scanning electron microscopy; PPAR, peroxisome proliferator-activated receptor; HSL, hormone-sensitive lipase; MRI, magnetic resonance imaging.

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pathies and obesity (4, 5, 18). For example, increased LPL in muscle and liver in Tg mice may lead to the over-production of FFA, accumulation of TG, and subsequent impairment of the insulin signaling, resulting in a state of insulin resistance (19). Therefore, it is still controversial, when LPL is within the physiological range, whether “the higher, the better or the lower, the better” holds in terms of systemic LPL activity in glucose metabolism and adipose accumulation. In addition, it remains unclear whether elevation of LPL can be used as a therapeutic method to treat hyperlipidemias, diabetes, and obesity or whether it is safe to use LPL-raising agents in hyperlipidemic patients with insulin resistance or diabetes.

To investigate the physiological functions of LPL in vivo, we generated Tg rabbits expressing human LPL (hLPL) and reported that overexpression of LPL has multiple effects on atherosclerosis and hyperlipidemias (14). Recently, we found that overexpression of LPL inhibited obesity in high fat diet-fed rabbits. Based on these observations, we proposed that increased LPL activity may increase insulin sensitivity and subsequently improve insulin resistance in hyperlipidemic and diabetic states. In this study, we cross-bred hLPL Tg rabbits with LDL receptor-deficient Watanabe heritable hyperlipidemic (WHHL) rabbits, a model of human familial hypercholesterolemia (20). WHHL rabbits developed spontaneous hypertriglyceridemia, hypercholesterolemia, atherosclerosis, and myocardial infarction accompanied by hyperinsulinemia (21, 22), which may mimic the features of metabolic syndrome in humans. We hypothesized that overexpression of LPL may improve hyperlipidemia and insulin resistance in WHHL rabbits. The current study using LPL Tg WHHL rabbits was designed with two goals in mind: (i) to study the effects of increased LPL activity on both hypertriglyceridemia and hypercholesterolemia and (ii) to determine whether high LPL would influence insulin sensitivity and adiposity in the absence of LDL receptor functions. We found that increased LPL activity essentially corrected hypertriglyceridemias in WHHL rabbits but had a gender-dependent effect on cholesterol metabolism and fat accumulation.

MATERIALS AND METHODS

Generation of Human LPL Transgenic WHHL Rabbits—The generation of Tg rabbits expressing hLPL under the control of the chicken β-actin promoter has been described previously (14). In this study, hemizygous LPL Tg rabbits were bred with the same colony of homozgyous WHHL rabbits used in the previous study (23). By sequential breeding, we obtained two groups of Tg WHHL rabbits differing in their LDL receptor (LDLr) status: heterozygous Tg WHHL rabbits (hLPL/LDLr⁻/⁻) and homozgyous Tg WHHL rabbits (hLPL/LDLr⁻/⁻). LDLr genotype status was examined by PCR method developed by Broussseau et al. (24). The presence of the hLPL transgene was confirmed by PCR analysis using the primers 5'-CAT TGC AGG TCT AGG C-3' and 5'-GGA TTC CAA TGG TTC GAC C-3'. All animal experiments were performed with the approval and according to the guidelines of the Animal Research Committee of the University of Tsukuba.

Northern Blot Analysis—Total RNA was isolated from adipose tissues and skeletal muscles of Tg and non-Tg WHHL rabbits. Ten micrograms of RNA was denatured in the presence of dimethyl sulfoxide and glyoxal and was then subjected to electrophoresis in a 1.2% agarose gel and transferred to a Nytran nylon membrane (Schleicher &Schuell). The membrane was hybridized in turn with the 32P-labeled hLPL, rabbit LDLr, rat PPAR-γ2 and -δ, and hormone-sensitive lipase (HSL) cDNA probes (14). The blots were scanned using an imaging densitometer (Bio-Rad). Analysis of LPL Protein and Enzymatic Activity—Post-heparin plasma was prepared from a blood sample taken 10 minutes after a bolus injection of heparin at a dose of 30 units/kg of body weight. The amount of LPL protein mass was determined using an enzyme-linked immunosorbent assay (25). The enzymatic activity of LPL was determined using a 14C-labeled triolein emulsion substrate as described (14).

Plasma Lipid and Lipoprotein Analysis—The plasma lipid and lipoprotein profiles of Tg WHHL rabbits were compared with those of age- and sex-matched littermates. Blood was collected from rabbits after 16 h of food deprivation. Plasma total cholesterol (TC), TG, HDL-C, and FFA were determined using Wako assay kits (26).

Analysis of Glucose Metabolism and Insulin Sensitivity—To evaluate the effect of LPL on glucose metabolism, rabbits were subjected to a fasting overnight and then, the intravenous glucose tolerance test (IVGTT) and intravenous insulin tolerance test (IVITT) were performed using the method developed by Zhang et al. (27). For IVGTT experiments, rabbits were intravenously injected with glucose solution (0.6 g/kg of body weight), and then blood samples were drawn at 5, 10, 15, 20, 30, 45, 60, 75, and 120 minutes. Blood glucose levels were determined by glucometer, and FFA levels were measured using glucose and FFA assay kits (Wako) and an insulin enzyme-linked immunosorbent assay kit (Shibayagi Co., Gunma, Japan). For IVITT experiments, blood was collected at 15, 30, 45, 60, 90, and 120 min after intravenous injection of insulin (Shimizu Pharmaceutical Co., Shizuoka, Japan) (1 unit/kg of body weight).

Quantitative Analysis of Adipose Tissue—Rabbits at 11 months of age were sacrificed, and adipose tissues from the subcutaneous (inguinal and interscapular adipose) and visceral areas (abdominal cavity, mesenterium, and retroperitoneal adipose) were collected and their wet weight measured. The data were expressed as total body fat weight (g) and percentage of body weight. In addition, we fixed adipose tissue in 10% neutral-buffered formalin, postfixed it in 1% osmium tetroxide, and observed it using a scanning electron microscope (SEM) (JEOL, JSM-6320F, Tokyo, Japan). Random areas of each part of the adipose tissues was observed under 200-fold magnification and photographed, and then the diameter of the adipocytes was determined by the method described by Sugihara et al. (28).

High Fat Diet Experiment—To investigate the role of LPL on the high fat (HFD)-induced obesity and concurrent insulin resistance, 6 Tg heterozygous WHHL and 5 non-Tg littermates aged 6 months were fed a diet containing 10% fat (6.7% corn oil and 3.3% lard) for 14 weeks. IVGTT was performed at the start and the end of the experiment. Body weight was measured directly after HFD feeding. For the analysis of the HFD food consumed by each rabbit per day was monitored from 5 to 13 weeks every 2 weeks. Rabbits were sacrificed at 14 weeks of HFD, and adipose tissues were collected and measured as described above. For the evaluation of adipocyte size, the adipose tissues were fixed in 10% neutral-buffered formalin and observed by SEM as described above.

Estrogen Studies—To elucidate the possible mechanism giving rise to the gender-related phenotypes observed in Tg WHHL rabbits (see “Results”), the effect of sex hormones on plasma lipids and LPL activity was investigated in both male and female LPL Tg rabbits. For the experiment using male rabbits, 3 male Tg WHHL wild-type rabbits and 3 age-matched non-Tg littermates at 6 months of age were injected intramuscularly with 17α-ethinyl estradiol (Sigma) at a dose of 100 μg/kg/day for 10 days (29). After 0, 5, and 10 days of estrogen treatment, pre-heparin plasma was collected for plasma lipid analysis. Post-heparin plasma for determination of LPL activity was collected 10 min after a bolus injection of heparin at a dose of 30 units/kg of body weight at 0 and 10 days of estrogen treatment. For the experiment with female rabbits, 3 Tg wild-type rabbits and 3 heterozygous Tg WHHL rabbits along with their age-matched non-Tg littermates underwent a bilateral ovariectomy at 6 months (30). LPL activity and the mass of postheparin plasma were determined at 0 and 10 days after surgery.

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

RESULTS

Successful cross-breeding between hLPL Tg rabbits and WHHL rabbits was confirmed by the PCR analysis shown in Fig. 1A. Northern blot analysis revealed that high levels of hLPL expression were detected in muscles and adipose tissues of Tg WHHL rabbits, whereas rabbit endogenous LPL was mainly found in adipose tissues of both Tg and non-Tg WHHL rabbits (Fig. 1B). Overexpression of hLPL transgene in Tg WHHL rabbits resulted in a 3-fold (male) and 2-fold (female) increase of post-heparin plasma LPL activity compared with that in non-Tg WHHL rabbits (Table I). LPL activity in pre-heparin plasma of Tg WHHL rabbits was also increased (Table

2 S. Kitajima, M. Morimoto, T. Koike, and J. Fan, unpublished observations.
amounts of RNA had been loaded in each lane (lower). LDLr panel lower middle panel phoresis after PCR amplification. PCR products were either directly WHHL rabbits was determined by 12% polyacrylamide gel electro-

blot analysis of hLPL and endogenous rabbit LPL in adipose tissues (inguinal, visceral, and interscapu-

tates from adipose tissues (inguinal, visceral, and interscapu-

Lipoprotein Composition—unbound hLPL protein in the circulation. Their TG levels were close to the mean TG levels of normal females; significantly lower than control) but it did not reach the level of statistical signif-

A. PCR analysis

FIG. 1. Detection of hLPL transgene and determination of LDL receptor status in Tg WHHL rabbits by PCR (A) and Northern blot analysis of hLPL and endogenous rabbit LPL in adipose and skeletal muscle tissues (B). A, hLPL transgene was detected by PCR analysis as a 173-bp band (upper panel). Mutation of LDLr in WHHL rabbits was determined by 12% polyacrylamide gel electro-

Effect of Increased LPL Expression on Plasma Lipids and Lipoprotein Composition—As summarized in Table II, male Tg WHHL rabbits showed a marked reduction of plasma lipids (58 and 86% reductions of plasma TC and TG compared with those of non-Tg WHHL littersmates at 9 months of age, respectively). Their TG levels were close to the mean TG levels of normal Japanese white rabbits in our facility. In female Tg WHHL rabbits, plasma TG levels were decreased by 56%; however, the TC levels remained unchanged compared with those in non-Tg WHHL rabbits (Table II). HDL-C levels were slightly elevated in both male and female Tg WHHL rabbits. FFA contents were lower in Tg WHHL rabbits than non-Tg WHHL rabbits, but fasting glucose and insulin levels were not changed.

Plasma lipoproteins from male Tg WHHL and non-Tg WHHL rabbits were separated by sequential density gradient ultracentrifugation and analyzed as previously described (14). The major changes in the lipoprotein profiles of Tg WHHL rabbits included remarkably low levels of VLDL (d < 1.006 g/ml) and intermediate density lipoproteins (IDL) (d = 1.006–1.02 g/ml) associated with a relative increase of the LDL cholesterol level (data not shown).

Effect of Increased LPL Activity on Insulin Resistance—To investigate the effect of LPL on insulin sensitivity, 9-month-old rabbits were subjected to fasting overnight and intravenously injected with glucose for IVGTT. As shown in Fig. 2, there was no significant difference between male and female Tg WHHL rabbits in terms of the clearance rate of glucose and secretion of insulin compared with their non-Tg WHHL rabbits. The clearance rate of FFA was, however, enhanced in both sexes of Tg WHHL rabbits as their FFA levels were constantly and significantly lower than those of non-Tg WHHL rabbits (Fig. 2, A and B). In addition, we analyzed the insulin resistance (IR) index using a modified method developed by Mukherjee et al. (31), which reflects the amount of glucose and insulin levels under the conditions of the glucose tolerance test. We found that there was no difference between Tg and non-Tg WHHL rabbits (Fig. 2, A and B). Furthermore, we performed IVITT to examine the direct response to insulin in LPL Tg WHHL rabbits. However, we did not find any differences between the two groups (Fig. 2C). Taken together, these results indicate that systemic overexpression of LPL activity did not alter insulin sensitivity in Tg WHHL rabbits fed a chow diet.

Increased LPL Activity Reduced the Fat Tissue—At 11 months of age, all rabbits were sacrificed for analyses of body adipose tissues. For determination of body fat, subcutaneous and visceral fat were carefully collected and weighed while wet. As shown in Fig. 3A, male Tg WHHL rabbits showed a significantly lower amount of subcutaneous and visceral adipose tissues than non-Tg WHHL littersmates, whereas body weights were similar. Total amounts of adipose tissue in male Tg WHHL rabbits were reduced by 61% compared with those in non-Tg WHHL rabbits. Magnetic resonance imaging (MRI) examination of male Tg WHHL rabbits aged 6 weeks also revealed that these rabbits had less visceral adipose tissue than non-Tg littersmates (Fig. 3C). In female Tg WHHL rabbits, overexpression of LPL led to a slight reduction of body adipose (12% less than control) but it did not reach the level of statistical significance (Fig. 3B). To determine the changes in adipocytes of male Tg WHHL rabbits, we examined cellular morphology using SEM and determined cellular size and distribution. As shown in Fig. 4 (A and B), both subcutaneous and visceral adipocytes of Tg WHHL rabbits were predominantly composed of small-sized...
adipocytes and their average diameter was much smaller than that in non-Tg WHHL rabbits: 101.4 ± 2.7 non-Tg versus 81.5 ± 2.1 Tg (p < 0.01), in subcutaneous adipose and 82.1 ± 1.9 non-Tg versus 70.4 ± 1.1 μm Tg (p < 0.01); in visceral adipose tissue, respectively. These data indicate that the reduction of adipose tissue in Tg WHHL rabbits was associated with both reduction of the total number of adipocytes and of the cellular size. To explore the possible mechanism of the reduced adipose accumulation in Tg WHHL rabbits, we examined the expression of PPAR-γ2, a transcription factor for lipogenesis; PPAR-α, a transcription factor for lipolysis (β-oxidation of FFA); and HSL, an enzyme for hydrolysis of TG in adipose tissue using Northern blots. As shown in Fig. 5, the expression of these genes was not altered in the adipose tissue of Tg WHHL rabbits, suggesting that these factors are not responsible for the leanness seen in Tg WHHL rabbits.

High Fat Diet Experiments—To further elucidate LPL effects on insulin resistance and obesity, male heterozygous Tg WHHL rabbits were fed a HFD for 14 weeks and their body weight (BW) gain, body fat composition, and glucose metabolism were compared with those of non-Tg WHHL rabbits. As shown in Fig. 6A, Tg WHHL rabbits had a similar BW to non-Tg WHHL rabbits on a chow diet, but constantly gained less BW than did non-Tg WHHL rabbits fed HFD. We excluded the possibility that the lower BW gain in Tg WHHL rabbits was caused by less food consumption. The total daily amounts of HFD consumed by Tg WHHL rabbits were basically identical to those consumed by non-Tg WHHL rabbits but slightly higher at 7, 9, and 13 weeks when calculating by BW (Fig. 6B), suggesting that LPL may increase the expenditure rate in Tg WHHL rabbits. HFD feeding elevated plasma insulin and FFA levels in non-Tg but not in Tg WHHL rabbits (Fig. 6C).

To investigate the effect of overexpression of LPL on glucose metabolism in HFD fed-rabbits, we performed IVGTT on these animals. As shown in Fig. 7, HFD feeding induced insulin resistance in non-Tg WHHL rabbits, as indicated by the fact that the clearance rate of plasma glucose was delayed and fact that the clearance rate of plasma glucose was delayed and
lin and FFA were constantly lower than those of non-Tg WHHL rabbits. This notion was further strengthened by calculating the IR index (Fig. 7), which showed that there was only a 202% increase of IR index in Tg WHHL rabbits compared with a 607% increase in non-Tg WHHL rabbits after HFD feeding. Finally, we sacrificed these HFD-fed rabbits and examined the subcutaneous and visceral adipose tissues. Both net adipose tissues and fat percentage of BW were significantly lower in Tg WHHL rabbits than those in non-Tg WHHL rabbits (Fig. 8). Hypertrophic adipocytes induced by HFD were examined by SEM. As shown in Fig. 9, both subcutaneous and visceral adipocytes were significantly smaller in Tg WHHL rabbits than in non-Tg WHHL rabbits (subcutaneous: 102.7 μm² in non-Tg versus 81.4 μm² in Tg; visceral: 96.7 μm² in non-Tg versus 81.0 μm² in Tg, p < 0.01), suggesting that overexpression of LPL inhibits HFD-induced adipocytic hypertrophy. The expression of HSL in adipose tissue was reduced in Tg WHHL rabbits compared with that in non-Tg rabbits, whereas PPAR-γ2 and -6 were not changed (Fig. 10).

**Estrogen Studies on LPL Activity and Plasma Lipids**—To investigate the potential role of sex hormones in modulating LPL functions and to explain the gender differences seen in Tg WHHL rabbits, male rabbits were injected with 17β-estradiol and female rabbits underwent bi-ovariectomy. After 10 days of injection of estrogen in males, as shown in Table III, LPL activity in post-heparin plasma of both Tg and non-Tg rabbits was reduced by 30 and 40% but lipid levels were not changed (data not shown). In females, however, after 10 days of ovariectomy, LPL activity remained unchanged in both Tg and non-Tg rabbits. In addition, the LPL mass of Tg and non-Tg rabbits was also not changed.
DISCUSSION

The current study was undertaken to test the feasibility of the idea that genetic manipulation of LPL activity would be useful for the treatment of hyperlipidemia arising from LDLr deficiency. Using WHHL rabbits, we demonstrated here that systemic overexpression of LPL ameliorated hypertriglyceridemia and reduced fat accumulation. The plasma TG-lowering effect of LPL appears to result from marked reductions of VLDL and IDL. In addition, overexpression of LPL also reduces hypercholesterolemia in male but not in female Tg WHHL rabbits. In male Tg WHHL rabbits, total plasma cholesterol levels were reduced by 58% as a result of the reduced amounts of VLDL- and IDL-cholesterol. This may be caused by the fact that increased LPL activity promotes the hepatic clearance of these particles, apparently independently of the LDLr pathway (32, 33). LPL may stimulate cellular uptake of lipoproteins through lipolysis, thereby facilitating the increased affinity of lipoproteins for cellular receptors (34–36). Second, it is also possible that LPL on the lipoprotein surface may directly modulate binding to the cellular receptors through a so-called non-catalytic bridging effect (33, 37).

Because WHHL rabbits also have hyperinsulinemia associated with insulin resistance (21), we envisioned that overexpression of LPL may improve insulin sensitivity in WHHL rabbits. Conflicting results have been reported in the previous studies on Tg mice as to whether increased muscle and liver LPL expression induces insulin resistance by causing defects in insulin signaling and action (19, 38, 39). LPL gene transfer by adenovirus into LPL-deficient mice or cats (9, 40) did not result in impaired glucose tolerance or an insulin-resistant state.3 In rats and rabbits, administration of an LPL activator, NO-1886, suppresses fat accumulation and insulin resistance (15, 16). In support of the latter notion, we found that, in Tg WHHL rabbits...

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3 G. Liu, personal communication.
bits on a chow diet, overexpression of LPL reduces body fat accumulation (significantly only in males) without impaired glucose tolerance or insulin sensitivity. The beneficial effects of LPL on body fat accumulation and insulin sensitivity were further corroborated by the HFD feeding experiment on heterozygous WHHL rabbits. Apparently, overexpression of LPL in WHHL rabbits showed protection against HFD-induced fat accumulation and insulin resistance. The molecular mechanism(s) accounting for this observation is currently unknown; however, such beneficial effects are not caused by the dysfunctional LDLr because the same results were also obtained in wild-type (normal) LPL Tg rabbits. LPL hydrolyzes TG-rich lipoproteins, resulting in the release of FFA, which are either taken up and used for metabolic energy in peripheral tissues such as muscle and liver or reesterified into TG and stored in adipose tissue. The balance of these competing effects could determine whether increased LPL activity will lead to a reduced rate of weight gain (through greater disposal of ingested fats as metabolic fuel) or increased adiposity through increased rates of adipose storage of TG. Tg WHHL rabbits consumed a similar amount of food, and the basal temperature (data not shown) was the same as their counterparts. Furthermore, Tg WHHL rabbits expressed relatively higher levels of LPL in muscle than in adipose tissues (Fig. 1B). Therefore, we speculated that systemic overexpression of LPL may lead to increased energy expenditure in Tg WHHL rabbits. To prove this contention, however, it will be necessary to investigate whether Tg WHHL rabbits have decreased respiratory quotient (i.e. increased lipid oxidation) or increased oxygen consumption (i.e. increased energy expenditure) in the future.

In IVGTT experiments, FFA was cleared faster in Tg than non-Tg WHHL rabbits, possibly because of enhanced uptake of FFA in peripheral (non-adipose) tissues associated with enhanced \( \beta \)-oxidation. In support of this notion, a recent study showed that LPL is a key enzyme for the generation of PPAR-\( \alpha \) ligands (41), thereby promoting \( \beta \)-oxidation and ketogenesis. In Tg WHHL rabbits, adipocytes consist predominantly of a smaller sized population than those in non-Tg WHHL rabbits.
This phenomenon can be explained by assuming that systemically increased LPL activity decreases fat accumulation by accelerating the lipolytic process or inhibiting de novo lipogenesis, which was caused by competitively increased uptake of FFA by non-adipose tissue such as muscle, although this hypothesis remains to be verified. Alternatively, LPL may regulate or interact with other proteins that are associated with lipogenesis and/or lipolysis in the adipocytes such as HSL and PPAR-γ2 or PPAR-δ. It has been reported that LPL stimulates HSL expression in the adipose tissue of Tg mice as a compensatory mechanism for the inhibition of fat accumulation (42); however, we found that HSL expression was reduced in Tg WHHL rabbits as shown by Northern blotting analysis (Figs. 5 and 10). Nevertheless, it is generally believed that smaller size adipocytes found in Tg WHHL rabbits suggest that LPL may be a therapeutic target for the treatment of diabetes and obesity.

A noteworthy finding in this study was the significant gender difference in Tg WHHL rabbits, with males having reduced TC levels and less fat accumulation. Such a gender-dependent effect of LPL on lipoprotein metabolism and fat accumulation in Tg WHHL rabbits may be attributed to the lower post-heparin LPL mass and activity in females compared with males. Nevertheless, it is generally believed that smaller size adipocytes is associated with improved insulin sensitivity (43). If this is the case, the reduced adipocyte size, along with lower levels of plasma FFA in Tg WHHL rabbits may help to explain why Tg WHHL rabbits have increased insulin sensitivity compared with non-Tg WHHL rabbits when fed a HFD.

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