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High lipoprotein lipase activity increases insulin sensitivity in transgenic rabbits

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Abstract

Lipoprotein lipase (LPL) is the rate-limiting enzyme in the hydrolysis of triglyceride-rich lipoproteins and plays an important role in glucose metabolism. To examine the hypothesis that increased LPL activity may alter insulin sensitivity, we investigated glucose metabolism and insulin sensitivity in transgenic (Tg) rabbits expressing the human LPL gene under the control of a β-actin promoter. An intravenous glucose tolerance test showed that the plasma glucose clearance rate was not significantly different between Tg and non-Tg rabbits; however, the area under the curve for insulin and free fatty acids in Tg rabbits was significantly reduced compared with that of non-Tg rabbits ($P < .05$). Using the intravenous insulin tolerance test, we found that the area of under the curve of glucose of Tg rabbits was also significantly reduced ($P < .01$). Furthermore, euglycemic-hyperinsulinemic clamp test revealed that the mean glucose infusion rate in Tg rabbits was significantly higher than in non-Tg rabbits ($P < .05$). These results demonstrate that systemic overexpression of LPL increases whole-body insulin sensitivity and genetic manipulation of LPL genes may be a potential target for the treatment of diabetic patients.

1. Introduction

Lipoprotein lipase (LPL) is the rate-limiting enzyme for the hydrolysis of triglyceride (TG)-rich lipoproteins. Lipoprotein lipase is expressed in a variety of tissues, mainly adipose, skeletal, and cardiac muscle [1,2]. Lipoprotein lipase is first synthesized in the tissues and transported to the surface of endothelial cells of the capillary beds, where LPL hydrolyzes chylomicrons and very low-density lipoproteins to release free fatty acids (FFAs), generates remnant lipoproteins, and gives rise to the formation of high-density lipoproteins (HDL) [1]. Free fatty acid is either metabolized as energy or stored as TGs [3].

Abnormalities in LPL functions have been found to be associated with a number of pathophysiological processes such as hyperlipidemia, atherosclerosis, obesity, and insulin resistance (IR) [4]. Genetic LPL deficiency in human beings is associated with impaired insulin sensitivity [5]. However, some studies using transgenic (Tg) mice showed that increased LPL activity in the muscle and liver could induce IR. For example, Ferreira and coworkers [6] and Pulawa and Eckel [7] showed that human LPL Tg mice exhibited a state of IR, presumably because of increased delivery of lipoprotein-derived fatty acids to muscle, a major site of insulin-stimulated glucose disposal, whereas Kim et al [8] proposed a direct and causative relationship between the accumulation of intracellular fatty acid–derived metabolites and IR via alterations in the insulin signaling pathway in mice. In spite of this, Voshol and associates [9] found that overexpression of human LPL in mouse muscle increased TG accumulation in the

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muscle, but did not affect whole-body or muscle-specific insulin-mediated uptake, which argues against a simple causal relationship between intramuscular TG content and IR. The mechanisms for the effect of FFA and TG on insulin-stimulated glucose transport are complex, and whether increased LPL activity can improve insulin sensitivity is yet to be defined.

Our laboratory generated Tg rabbits expressing human LPL transgene and reported that overexpression of LPL protected against diet-induced hypercholesterolemia and atherosclerosis [10]. Features of lipoprotein metabolism in rabbits are very similar to those in human beings but are unlike those of murine metabolism. Thus, LPL Tg rabbits are adequate models for the study of the cause-and-effect relationship between LPL and IR. Furthermore, several lines of evidence have shown that even expression of the same transgene in Tg mice and rabbits may result in completely different finding [11]. Recently, we demonstrated that increased LPL expression improves hyperlipidemia and obesity in Watanabe heritable hyperlipidemic (WHHL) rabbits, which are deficient in low-density lipoprotein (LDL) receptor functions [12]. In this study, we attempted to examine the hypothesis that increased LPL activity may alter the whole-body insulin sensitivity in normal rabbits.

2. Materials and methods

2.1. Animals

Human LPL Tg rabbits were generated by microinjection of human LPL cDNA under the control of chicken β-actin promoter as described previously [10]. For the current studies, male LPL Tg rabbits (F1 hemizygous rabbits from L17 line) mated with normal female Japanese white rabbits (JW:kbt, Biotek Co, Saga, Japan). Hemizygous male Tg rabbits (LPL gene$^{+/0}$) and nontransgenic (non-Tg) littermates were used. Genotypes were identified from ear biopsy DNA by polymerase chain reaction analysis as reported [10]. All rabbits were kept individually in a room where the temperature and humidity were maintained at 24 $\pm$ 2°C and 55 $\pm$ 15%, respectively. The rabbits were submitted to a regular 12-hour light/dark cycle and given water and a standard chow diet (CBR-1, CLEA Inc, Tokyo, Japan) ad libitum. All animal protocols were approved by the Saga University Animal Experimentation Committee and performed under the Saga University Guidelines for Animal Experimentation.

2.2. Analysis of LPL, plasma lipids, glucose, and insulin

Postheparin plasma was prepared from a blood sample taken 10 minutes after a bolus injection of heparin at a dose of 30 U/kg body weight. The activity of LPL protein in postheparin plasma was determined described previously [10]. The plasma lipid and lipoprotein profiles of LPL Tg rabbits were compared with those of age- and sex-matched non-Tg littermates. Blood was collected from rabbits after 16 hours of food deprivation. Plasma total cholesterol (TC), TG, HDL cholesterol (HDL-C), FFA, and glucose were measured using Wako assay kits (Wako Pure Chemical Industries, Osaka, Japan). The plasma insulin assay was conducted using enzymes-linked immunoassay insulin kits (Morinaga Bioscience Institute, Yokohama, Japan) with rabbit insulin as a standard.

2.3. Intravenous glucose tolerance test

The intravenous glucose tolerance test (IVGTT) was performed as previously described [13,14]. The animals were fasted overnight (16 hours), after which a bolus of glucose (0.6 g/kg body weight) was injected through the ear vein and blood samples were collected through the ear artery at 0, 5, 10, 15, 20, 30, 45, 60, 75, and 120 minutes. Plasma glucose, FFA, and insulin concentrations were measured as described above. The incremental area under the curve (AUC) was calculated according to the trapezium rule [15], and the IR index was calculated according to the method described by Mondon [16].

2.4. Intravenous insulin tolerance test

The intravenous insulin tolerance test (IVITT) was performed by injecting animals, which were fasted, with a bolus of insulin (1.0 U/kg body weight; Shimizu Pharmacy Inc, Shizuoka, Japan) through an ear vein; blood was drawn and analyzed as above.

2.5. Euglycemic-hyperinsulinemic clamp studies

Insulin sensitivity was assessed using the euglycemic-hyperinsulinemic clamp procedure first described by DeFronzo et al [17] and adapted to rabbits [18] with slight modifications. Briefly, rabbits were fasted for 16 hours and were subjected to pentobarbital sodium anesthesia (40 mg/kg body weight IV). An indwelling catheter was then inserted into the jugular vein for simultaneous administration of glucose and insulin. Once the basal plasma glucose levels were stable, soluble insulin (Shimizu) dissolved in 0.9% saline containing 3% bovine serum albumin was continuously administered for 120 minutes at 0.75 mU/(kg·min) using a Terumo Syringe Pump STC-525 (Terumo Corporation, Tokyo, Japan). Four minutes after insulin infusion, 10% glucose was infused from 4 to 120 minutes using a KD Scientific Syringe Pump KDS-200 (KD Scientific Inc, Holliston, MA). The plasma glucose levels were maintained at basal preinfusion levels throughout the changing of the glucose infusion rate (GIR). The GIR was adjusted according to plasma glucose concentrations, which were measured at 5-minute intervals using 50-μL blood samples collected from an ear artery by a Glucose/L-Lactate Analyzer YSI 2300 STAT (Yellow Springs Instruments, Yellow Spring, OH). Clamp GIR was calculated from the GIR obtained during last 30 minutes of clamping.

2.6. Insulin signaling assay

Fasted animals at 40 weeks of age were anesthetized, and the skeletal muscle (soleus) from the left hindlimb was collected. The rabbits were killed 10 minutes after injection of a bolus of insulin (1.0 U/kg body weight, Shimizu) through the ear vein, and the soleus muscle from the right hindlimb was collected. Samples were immediately frozen in liquid nitrogen after excision and stored at −80°C for late insulin receptor substrate 1 (IRS-1) analysis. A piece (500 mg) of the skeletal muscle (soleus) was homogenized in an ice-cold lysis buffer (1:8 wt/vol) containing 20 mmol/L Tris-HCl, 5 mmol/L EDTA, 10 mmol/L $Na_4P_2O_7$, 100 mmol/L NaF, 2 mmol/L Na_3VO_4, 1% NP-40, 10 μmol/L leupeptin, 3 mmol/L benzamidine, 10 μg/mL aprotinin, 1 mmol/L phenylmethylsulfonylfluoride, then centrifuged at 13 000*g* for 10 minutes at 4°C. The supernatants were collected, and protein concentrations were determined using a Bradford dye binding assay kit (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as a standard. For immunoprecipitation, aliquots (0.5 mg) of sample lysates were immunoprecipitated with polyclonal antibody against rat liver IRS-1 (Upstate Biotechnology Inc, Lake Placid, NY) coupled with ImmunoPure immobilized Recomb Protein A beads (Pierce Biotechnology, Inc, Rockford, IL) at 4°C. The beads were washed 3 times with the same buffer, resuspended in Laemmli sample buffer, and boiled for 5 minutes. Proteins were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Immunoblotting was performed using *p*-tyrosine monoclonal antibody (PY99, SC7020, Santa Cruz Biotechnology, Santa Cruz, Calif) overnight at 4°C, and proteins were detected by enhanced chemiluminescence (Amersham Pharmacia, Buckinghamshire, UK).

2.7. Quantitative analysis of adipose weight

Rabbits were killed at the age of 40 weeks, and the adipose tissues, including subcutaneous fat (inguinal and interscapular adipose) and visceral fat (abdominal cavity, mesenteries, and retroperitoneal adipose), were carefully collected and weighed while wet [12]. The data were expressed as fat weight (grams) and percent of body weight.

2.8. Statistical analysis

Results are expressed as means ± SE. Statistical analysis was performed using Student *t* test for comparison of unpaired data between groups. $P < .05$ was considered statistically significant.

3. Results

3.1. Effect of increased LPL expression on plasma lipids, lipoprotein composition, glucose, and insulin

Human LPL Tg rabbits had 3-fold higher LPL activity in postheparin plasma than non-LPL Tg rabbits (Table 1). Increased LPL expression in Tg rabbits led to a significant reduction of plasma lipids (75% in TG, 28% in TC, 48% in HDL-C, and 28% in FFA) compared with those of non-Tg littermates (Table 1). Plasma levels of glucose and insulin were not significantly different between Tg and non-Tg rabbits (Table 1).

3.2. Effect of increased LPL activity on insulin sensitivity

To investigate the effect of increased LPL activity on whole-body insulin sensitivity, rabbits were fasted overnight and were intravenously injected with either glucose (IVGTT) or insulin (IVITT). As shown in Fig. 1A, there was no significant difference between Tg and non-Tg rabbits in terms of the clearance rate of injected glucose. However, FFA levels in Tg rabbits were constantly lower (statistically significant at 45, 75, and 120 minutes) than that in non-Tg rabbits (Fig. 1B), which was accompanied by lower levels of plasma insulin (Fig. 1C), suggesting that insulin sensitivity in Tg rabbits was relatively enhanced. This notion was further strengthened by the analysis of AUC summarized in Table 2. We found that the AUC of FFA and insulin was significantly smaller in Tg rabbits than that in non-Tg rabbits. We then measured the IR index, which reflects the amount of insulin required for the same amount of glucose metabolism under the conditions of the glucose tolerance test. The IR index of Tg rabbits was also significantly lower than non-Tg rabbits ($P < .05$). To prove that Tg rabbits had high insulin sensitivity directly, we compared the direct response to insulin injection by IVITT in Tg and non-Tg rabbits. As shown in Fig. 1D, the glucose clearance rate in Tg rabbits was significantly faster (at all time points) than non-Tg rabbits (AUC of glucose values: 157.3 ± 7.3 in non-Tg vs 127.3 ± 5.3 in Tg, n = 10, $P < .01$). Taken together, these results indicate that systemic overexpression of LPL increases insulin sensitivity in Tg rabbits.

Table 1
LPL enzymatic activity in postheparin plasma and plasma levels of lipids, glucose, and insulin in Tg and non-Tg rabbits

	LPL [μmol/L FFA/(mL · min)]	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	FFA (mEq/L)	Glucose (mg/dL)	Insulin (ng/mL)
Tg (n = 10)	0.511 ± 0.026*	8.7 ± 1.6*	13.1 ± 1.7**	6.3 ± 0.8*	0.235 ± 0.031*	119.2 ± 5.4	0.70 ± 0.14
Non-Tg (n = 10)	0.158 ± 0.027	35.2 ± 3.2	18.1 ± 1.8	12.0 ± 1.1	0.328 ± 0.032	130.0 ± 7.4	0.66 ± 0.13

Results are expressed as the mean ± SE. Statistical significance was determined by Student *t* test. Rabbits were 29 to 32 weeks of age and were maintained on a chow diet, then fasted for 16 h before analyses.

* $P < .01$ vs non-Tg rabbits.

** $P < .05$ vs non-Tg rabbits.

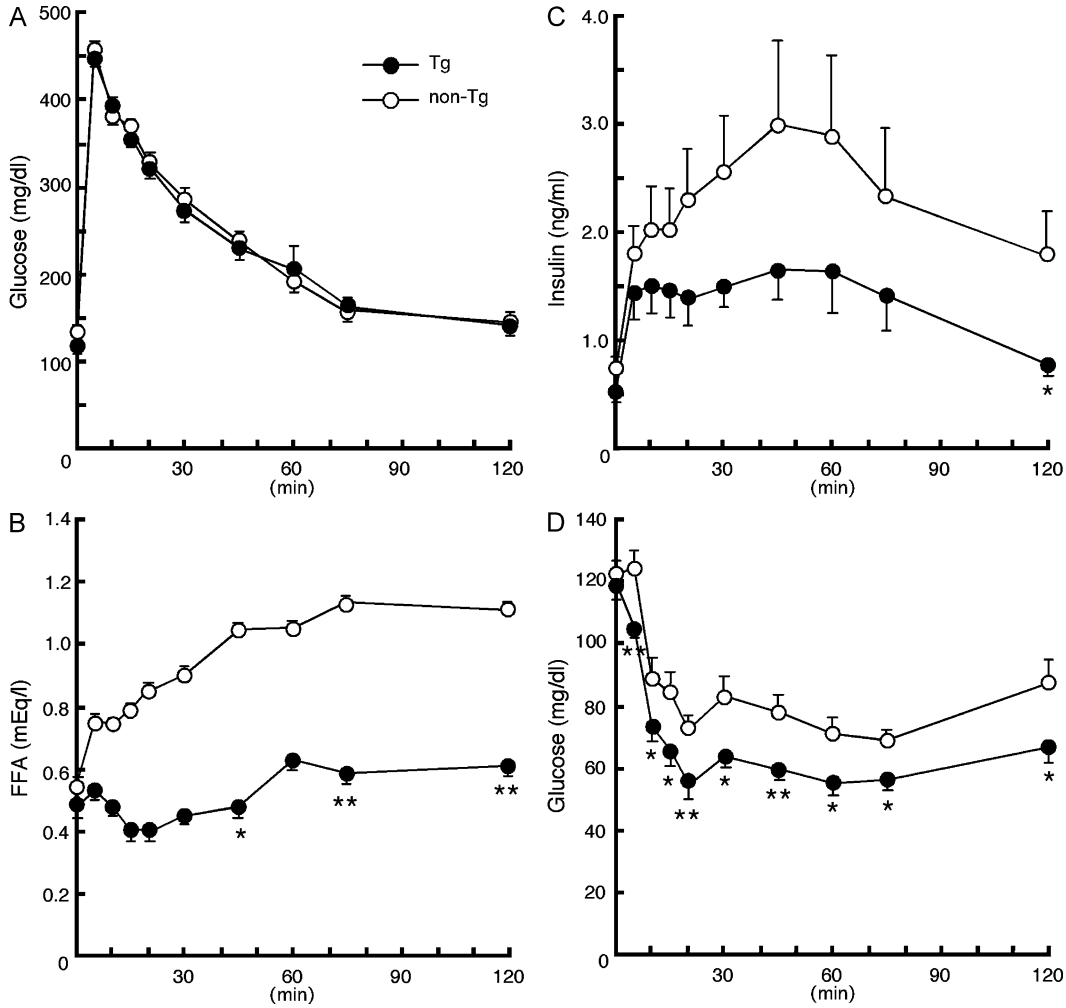

Fig. 1. Evaluation of glucose, FFA, and insulin metabolism after IVGTT (A, B, and C) and IVITT (D). Tg and non-Tg rabbits were injected intravenously with glucose solution or insulin, and plasma samples were collected as described under Materials and methods. The changes in plasma glucose, insulin, and FFA levels were determined and expressed as mean ± SE (n = 10 for each group). Animals were 29 to 32 weeks of age. *$P < .05$, **$P < .01$ vs non-Tg rabbits.

To examine the possibility that the faster FFA clearance and lower level of insulin secretion in Tg rabbits was caused by or associated with increased peripheral insulin sensitivity, we measured whole-body glucose utilization using an euglycemic-hyperinsulinemic clamp. Apparently, the mean GIR of Tg rabbits was higher than that of non-

Table 2
IVGTT, IVITT, and IR in LPL Tg and non-Tg rabbits

	AUC			IR index
	Glucose [mg/(dL · h)]	Insulin [ng/(mL · h)]	FFA [mEq/(L · h)]	
IVGTT				
Tg (n = 10)	441.3 ± 20.0	2.32 ± 0.31*	1.08 ± 0.2*	10.57 ± 1.42*
Non-Tg (n = 10)	449.6 ± 16.6	4.62 ± 0.85	2.02 ± 0.32	21.67 ± 4.30
IVITT				
Tg (n = 10)	127.3 ± 5.3**	ND	ND	ND
Non-Tg (n = 10)	157.3 ± 7.3	ND	ND	ND

Results are expressed as mean ± SE. The area of glucose, insulin, and FFA AUC was calculated by multiplying the cumulative mean height of glucose (mg/dL), insulin (ng/mL), and FFA (mEq/L), respectively, by the time (hours). The IR index was calculated from the product of the area of glucose multiplied by insulin, divided by 100. Statistical significance was determined by Student *t* test. Rabbits were 29 to 32 weeks of age at the time of analysis.
ND indicates not determined.

* $P < .05$ vs non-Tg rabbits.

** $P < .01$ vs non-Tg rabbits.

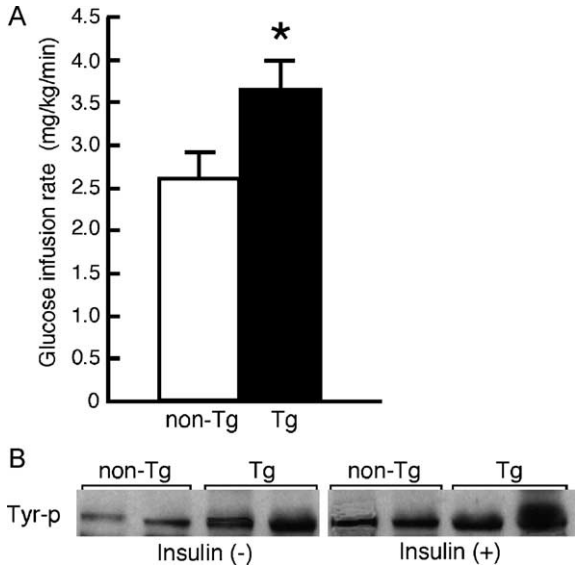

Fig. 2. Mean GIR after euglycemic-hyperinsulinemic clamps (A), insulin signaling in the skeletal muscles (B). Tg and non-Tg male rabbits were fasted for 16 hours and underwent blood glucose clamping under a constant insulin infusion rate of 0.75 mU/(kg · min). Mean GIRs in Tg rabbits were significantly higher than in their non-Tg counterparts (n = 5 for each group). *$P < .05$ vs non-Tg rabbits. Tyrosine phosphorylation of IRS-1 was analyzed as described in Materials and methods.

Tg rabbits (Fig. 2A), which was consistent with the above findings from IVGTT and IVITT.

3.3. Insulin signaling in Tg rabbits

The improved insulin response found in Tg rabbits led us to hypothesize that LPL may up-regulate insulin-stimulated glucose uptake and transport in skeletal muscles. We next compared the tyrosine phosphorylation of IRS-1 between Tg and non-Tg rabbits before and after insulin injection. As shown in Fig. 2B, tyrosine phosphorylation of IRS-1 in skeletal muscles was increased in Tg rabbits compared with non-Tg rabbits, both before and after insulin injection.

3.4. Reduced adipose tissue in Tg rabbits

We also investigated the effect of LPL expression on the body weight and adipose volume in Tg rabbits. As shown in Fig. 3, the body weight of Tg rabbits was slightly lighter (10.5% less over control) and their total fat tissue (especially visceral adipose) was significantly lower (39%) than non-Tg rabbits ($P < .05$). There was no difference in the daily food consumed between Tg and non-Tg rabbits (data not shown).

4. Discussion

The current study was undertaken to test the hypothesis that increased LPL activity would improve insulin sensitivity. In fact, there has been a controversial finding regarding LPL functions on IR. Transgenic mice studies showed that the enhancement of hepatic and muscle LPL may lead to the impairment of insulin signaling and subsequently give rise to IR [6,7]. However, some studies also provided evidence that increased LPL activity may be potentially beneficial. For example, Yin and Tsutsumi [19] reported that administration of an LPL-activator, NO-1886 in diabetic animals improved IR.

Using Tg rabbits expressing human LPL transgene, we demonstrated here that systemic overexpression of LPL improved whole-body insulin sensitivity. When challenged with an IVGTT, fasting plasma FFA and insulin responses in LPL Tg rabbits were significantly different from non-Tg littermates: they had lower levels of FFA and insulin associated with decreased AUC and IR indices. The AUC of glucose in Tg rabbits was more significantly reduced in IVITT, and euglycemic–hyperinsulinemic clamps at a mean GIR of Tg rabbits increased compared with non-Tg rabbits. Our results support the notion that increased LPL may be potentially beneficial for insulin sensitivity. To prove whether LPL may effectively improve IR or diabetic state, it is necessary to perform experiments using rabbits fed a high-fat or -glucose diet in the future. In a separate study, we found that LPL expression in Tg rabbits protected against high-fat diet (HFD)–induced IR and obesity [12,20] In human beings, the LPL gene deficiency is associated with impaired insulin sensitivity [4,5]. Therefore, one may envision that genetic manipulation of LPL expression may be a potential target to improve insulin sensitivity. In this aspect, Tsutsumi and coworkers

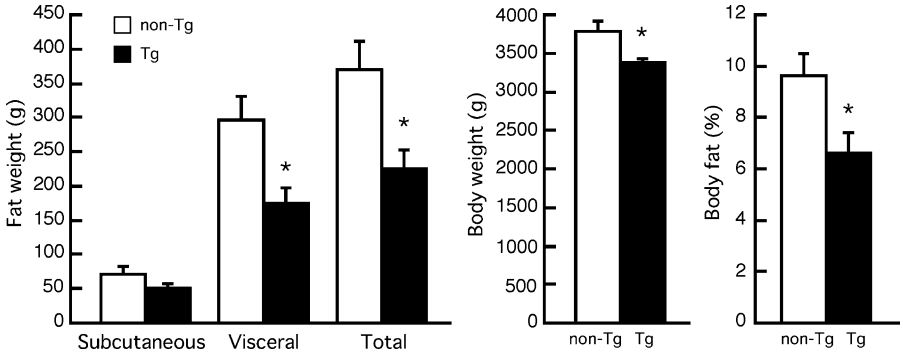

Fig. 3. Quantification of adipose tissue. Rabbits were killed at 40 weeks and their subcutaneous and visceral adipose tissues were collected and weighed. The results were expressed as mean ± SE (n = 6 rabbits for each group). *$P < .05$ vs non-Tg rabbits.

recently developed a new type of LPL activator that can induce muscle-specific expression and effectively correct IR in diabetic rats (personal communication), and Koike et al [12] demonstrated that overexpression of LPL effectively improves hyperlipidemia in WHHL rabbits in the setting of LDL receptor deficiency.

The possible molecular mechanisms for LPL-induced increase in insulin sensitivity are not fully understood, but are possibly attributed to the insulin signal pathway. Increased LPL may lead to enhancement of glucose uptake in the muscles via insulin-stimulated tyrosine phosphorylation of IRS-1 and, subsequently, result in the translocation of glucose transporter-4, although this hypothesis remains to be verified. Our study showed that IRS-1 levels in Tg rabbit muscles were higher than that in control rabbits. A recent study showed that LPL is a key enzyme for the generation of peroxisome proliferator–activated receptor-α ligands [21], thereby promoting β-oxidation and ketogenesis. Therefore, LPL may be indirectly involved in the glucose metabolism through the peroxisome proliferator–activated receptor pathway.

The present results obtained from Tg rabbits are in contrast to Tg mice studies [6-9]. Ferreira et al [6], Pulawa and Eckel [7], and Kim et al [8] reported that overexpression of human LPL in mice was associated with IR, whereas Voshol et al [9] found that LPL in Tg mice did not affect whole-body or muscle-specific insulin-mediated uptake. Lipoprotein lipase gene transfer by adenovirus into LPL-deficient mice or cats did not result in impaired glucose tolerance or an insulin-resistant state [22,23]. In rats and rabbits, administration of an LPL activator (NO-1886) significantly suppressed fat accumulation and IR [24,25]. Therefore, such discrepancies may lie in the species differences between mice and other mammals, such as rabbits and human beings, in terms of LPL functions on insulin response. For example, rabbit and human lipoproteins are rich in LDLs, whereas mice are HDL mammals; rabbits and human beings have cholesteryl transfer protein, whereas mice do not [11].

We found that overexpression of LPL in Tg rabbits significantly reduces body fat accumulation 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 experiment on LPL Tg rabbits [20] and WHHL rabbits [12]. Apparently, overexpression of LPL in rabbits also showed protection against HFD-induced fat accumulation and IR. We speculate that systemic overexpression of LPL may lead to increased energy expenditure in Tg rabbits.

In conclusion, our study demonstrated that systemic overexpression of LPL in rabbits increases insulin sensitivity. Although the molecular mechanisms are not fully defined, these findings suggest that genetic manipulation of LPL may be potentially effective for treating IR and diabetes.

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