Original Research

Glucose intolerance and decreased early insulin response in mice with severe hypertriglyceridemia

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Abstract

Hypertriglyceridemia (HTG) is one of the key features of dyslipidemia in type 2 diabetes, caused by the overproduction and/or decreased clearance of triglyceride (TG)-rich lipoproteins, and significantly promotes the development of cardiovascular diseases in diabetes. However, the effect of severe HTG on glucose metabolism has not previously been determined. Lipoprotein lipase (LPL) deficiency results in severe HTG in humans. By using LPL-deficient mice with severe HTG, we assessed the impact of severe HTG on insulin secretion and glucose tolerance in the present study. While young LPL-deficient mice (4 months of age) showed higher fasting blood glucose (7.42 ± 0.84 versus 4.8 ± 0.80 mmol/L, $P < 0.01$) and lower insulin concentrations (0.16 ± 0.03 versus 0.48 ± 0.14 ng/mL, $P < 0.05$), old mice (12 months of age) had higher insulin (1.70 ± 0.35 versus 0.77 ± 0.04 ng/mL, $P < 0.05$) but normal fasting blood glucose concentrations. Both young and old mice had elevated free fatty acid (FFA) concentrations and exhibited decreased early insulin response; however, only old mice showed impaired glucose tolerance, as compared with wild-type mice of a similar age. Morphological assessment showed enlarged islets in old LPL-deficient mice. These findings suggest that different tests for glucose homeostasis may be needed for patients with LPL deficiency and severe HTG, even though their glucose concentrations are normal at initial screening.

Keywords: lipoprotein lipase, glucose homeostasis, insulin secretion

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Introduction

Hypertriglyceridemia (HTG) and low high-density lipoprotein (HDL) are two prominent features of proatherogenic dyslipidemia in states of insulin resistance and type 2 diabetes mellitus.[1] HTG in type 2 diabetes is influenced by the level of glycemic control, nutritional state and genetic factors. Severe HTG is not uncommon in poorly controlled type 2 diabetic patients.[2] Owing to the central role of lipoprotein lipase (LPL, E.C.3.1.1.34) in hydrolyses of triglyceride (TG) in both chylomicrons (CMs) and very low-density lipoprotein (VLDL) particles and conversion of HDL, dysfunction of LPL could also result in dyslipidemia characterized by HTG and low HDL.[3,4] It has been reported that nearly 20% of patients with HTG carry common LPL gene mutations.[5]

Evidence indicates that patients with type 2 diabetes have a greater risk of developing atherosclerosis;[6] however, the significance of HTG as a risk factor for cardiovascular disease (CVD) has long been debated, and evidence of the clinical benefit from treating HTG remains unclear. Su and co-workers[7] reported impaired glucose tolerance (IGT) in 80 severe HTG patients with microalbuminuria, but the frequent coexistence of HTG with other risk factors such as obesity, insulin resistance and type 2 diabetes has made understanding the relationship between HTG and hyperglycemia more complex.[8] Until now, the effects that severe HTG might have on glucose tolerance or insulin secretion are still largely unknown.

The early stage of type 2 diabetes is characterized by postprandial hyperglycemia and reduced first-phase insulin secretion. Recently, ample evidence supports a new concept that postprandial HTG and hyperglycemia are both independent predictors of CVD.[9,10] On the other hand, it is known that insulin resistance and relative insulin deficiency in type 2 diabetic patients contribute to HTG through a series of mechanisms such as overproduction of

VLDL, decreased LPL activity, increased cholesteryl ester transfer protein activity and increased flux of free fatty acids (FFAs) to the liver.[11] In the present study we used a previously described LPL-deficient mouse model exhibiting severe HTG[12] to address the impact of severe HTG on glucose metabolism. In this investigation we assessed severe HTG as we believe mild or moderate forms of HTG may not uncover the underlying relationship between HTG and glucose metabolism.

In fact, the present study also holds clinical relevance given the incidence of severe HTG in China. As we know, familial HTG (FHTG) is an autosomal dominant disorder occurring in approximately one per 500 population.[13] In FHTG, the fasting serum TG concentrations range from 2.8 to 11.3 mmol/L.[13] That is to say, within the normal population a total of three million individuals displayed severe HTG in China.

Materials and methods

Animals

LPL-deficient mice of C57BL/6 background were rescued from neonatal death by intramuscular injection of an adenoviral vector coding a human LPL mutant, Ad-LPLS447X, as previously described.[12] Genotyping was performed by polymerase chain reaction (PCR). Adult mice were kept on a regular rodent diet and were divided into four groups with six animals per group. Because no sex difference in glucose concentrations was observed in pilot experiments, we kept mixed genders of half males and half females in each group. All mice were kept on a 12/12 h light/dark cycle with *ad libitum* access to water and chow. The principles of laboratory animal care (NIH publication no. 85–23, revised 1996) were followed, and the experimental protocol was approved by the Animal Care Committee, Peking University Health Science Center.

Glucose tolerance tests

Glucose tolerance tests (GTT) were performed as described previously.[14] Briefly, mice were fasted overnight for 14 –16 h, and then injected intraperitoneally with 2 g/kg body weight of glucose. Blood was collected from the tail vein at 0, 15, 30, 60 and 120 min. Glucose was measured using an automatic glucometer (One Touch Ultra, Lifescan, CA, USA).

Insulin secretion tests

In vivo analysis of insulin secretion was performed as described previously.[15] Briefly, mice were fasted overnight for 14–16 h, and glucose-stimulated insulin secretion (GSIS) was assessed following intraperitoneal injection of 3 g/kg body weight of glucose. Arginine-stimulated augmentation of GSIS (A + GSIS) was studied following intraperitoneal injection of 3 g/kg of glucose plus 0.3 mg/kg of arginine. Whole blood was collected by retro-orbital bleeds at 0, 2, 5 and 20 min (for GSIS) or 0, 2 and 5 min (for A + GSIS) and blood serum was stored at -20°C. Insulin was measured by ELISA (Linco Research Inc., Missouri, MO, USA).

Insulin tolerance tests

Insulin tolerance tests (ITT) were performed on fed mice with an intraperitoneal injection of 0.75 U/kg body weight of human regular insulin (Novodisk, Denmark). Whole blood was collected from the tail vein at 0, 15, 30 and 60 min and blood glucose was measured using an automatic glucometer (One Touch Ultra, Lifescan, CA, USA).

Plasma lipids

Blood was collected from overnight fasted mice by retro-orbital plexus bleeding. Total cholesterol (TC) and TG in plasma were analyzed enzymatically using commercial kits (Sigma) following manufacturer protocols. HDL-C was measured by measuring TC (Sigma) after ApoB-containing lipoproteins were precipitated with 20% polyethylene glycol solution. Serum FFAs were determined using the commercially available NEFA C kit with the enzymatic colorimetric method (Wako Chemicals, Osaka, Japan).

Pooled plasma lipoprotein profiles were generated by fast protein liquid filtration chromatography (FPLC) (Pharmacia LKB Biotechnology Inc, Piscataway, NJ, USA). Extremely hyperlipidemic plasma samples were centrifuged (30,000 rpm, 10 min) in an Optima™ TLX Ultracentrifuge (Beckman Coulter, Inc., Brea, CA, USA) and passed through a 0.2-μm filter before FPLC.

Histological observation

Pancreatic tissue was obtained from 12-month-old mice after overnight fasting. Tissue was fixed overnight in 10% formaldehyde, dehydrated in an ethanol bath and paraffin embedded. Tissue was cut into 10 sections (5 μm thick) separated by 200 μm. These sections were then stained with hematoxylin–eosin (H–E) and visualized using a microscope (Olympus, Tokyo, Japan) to enable observation of pancreatic tissue structure. At least five sections of three mice in each group were randomly selected and analyzed. The size of islets was measured by morphometric means (free NIH image J 1.41 Software).

Statistical analysis

The results were expressed as means $\pm$ SE. Statistical significance was tested using two-tailed Student's *t*-test to compare two groups. Differences between groups were regarded significant at $P < 0.05$.

Results

Body weight and lipid profile in severe HTG mice

In the present study, the LPL-deficient mice exhibited a decreased body weight compared to wild-type (WT) C57BL/6 mice (Figure 1a) and extreme HTG with a 40-fold increase in plasma TG versus WT mice. As expected, the LPL-deficient mice also displayed significant increases in plasma TC. Table 1 shows significantly elevated plasma FFA concentrations in both young and old LPL-deficient mice, as compared with the respective control groups (4.6 ± 0.4 versus 2.5 ± 0.3 and 4.6 ± 0.3 versus 2.5 ± 0.3 mEq/L,

both $P < 0.01$). As shown in Figure 1e the plasma lipoprotein profiles of these mice were consistent with previously reported findings in LPL-deficient mice,[12,16] characterized by an accumulation of large TG-rich lipoprotein particles.

Fasting glucose and insulin concentrations in severe HTG mice

Fasting insulin is a marker of insulin resistance in non-diabetic subjects.[17] In four-month-old (young) LPL-deficient mice, fasting insulin concentrations were initially lower than those of WT mice (0.16 ± 0.03 versus 0.48 ± 0.14 ng/mL, $P < 0.05$), but became significantly higher than WT mice (1.70 ± 0.35 versus 0.77 ± 0.04 ng/mL, $P < 0.05$) at 12 months of age (old) (shown in Figure 1b).

Young LPL-deficient mice showed a significantly higher fasting blood glucose compared with WT mice (7.42 ± 0.84 versus 4.8 ± 0.80 mmol/L, $P < 0.01$). At 12 months of age, fasting and non-fasting blood glucose concentrations were similar to WT mice (Figures 1c and d).

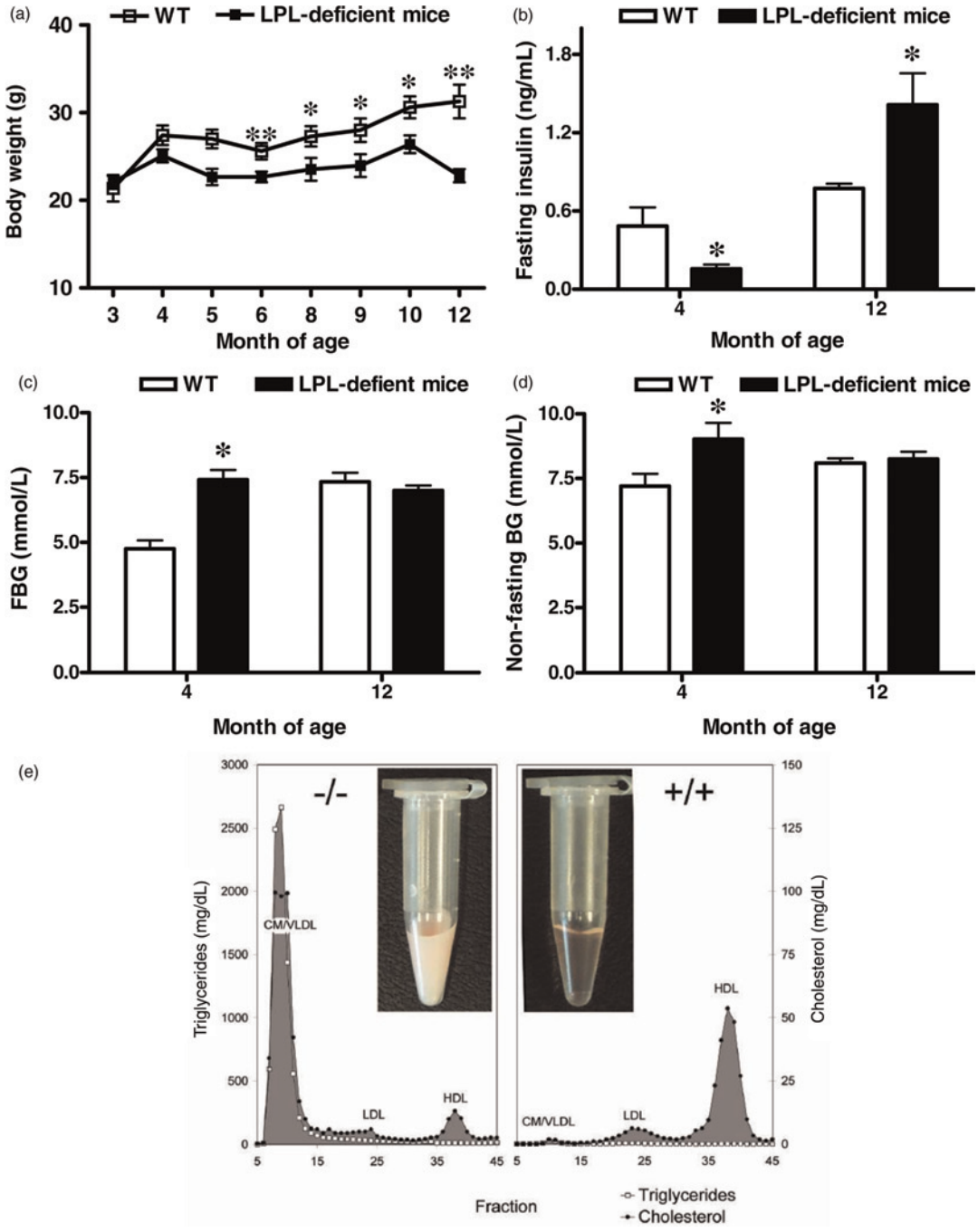

Figure 1 Growth curve, blood glucose, insulin levels in wild type (WT) and lipoprotein lipase (LPL)-deficient mice. (a) Growth curve. (b) Blood insulin was determined in fasted mice at four and 12 months of age. (c, d) Blood glucose was determined at four and 12 months of age in fasting (c) and non-fasting (d) mice. (e) The fast protein liquid filtration chromatography profile of four-month-old mice showed an increased CM/VLDL peak and a reduced HDL peak. $^{*}P < 0.05$, $^{**}P < 0.01$, LPL-deficient mice versus control. $N = 6$ per group. CM, chylomicron. (A color version of this figure is available in the online journal)

Table 1 Plasma lipids in LPL-deficient and WT mice

	4 months		12 months	
	LPL-deficient mice	WT	LPL-deficient mice	WT
TG (mmol/L)	163.2 ± 41.1	**3.5 ± 0.6	78.4 ± 14.7	**1.6 ± 0.2
TC (mmolL/)	5.3 ± 0.7	**1.3 ± 0.1	4.9 ± 0.7	**0.9 ± 0.1
HDL-c (mmol/L)	0.11 ± 0.021	**21.7 ± 0.16	0.026 ± 0.016	1.4 ± 0.11
FFA (mEq/L)	4.6 ± 0.4	**2.5 ± 0.3	4.6 ± 0.3	**2.5 ± 0.3

TG, triglyceride; TC, total cholesterol; FFA, free fatty acid; LPL, lipoprotein lipase; WT, wild type
Plasma TC, triglyceride, HDL-c levels and serum FFAs were determined by enzymatic methods. $^{**}P < 0.01$, lipoprotein lipase-deficient mice versus control. $N = 6$ per group

These results were indicative of defective insulin secretion in young LPL-deficient mice and insulin resistance in aged mice. The presence of reduced insulin secretion and insulin resistance are strongly associated with individuals who exhibit impaired fasting glucose and/or glucose tolerance, factors that are more likely to progress to diabetes.[18] Defective insulin secretion might result in excessive fasting hepatic glucose production accounting for fasting hyperglycemia in young LPL-deficient mice, while hepatic and/or muscle insulin resistance plays a more central role during old age.

Overall, the fasting hyperglycemia and fluctuation in fasting insulin concentrations suggest that severe HTG of LPL-deficient mice significantly affected the glucose metabolism and insulin-resistant status.

GTT and insulin secretion tests in severe HTG mice

The ability of WT and LPL-deficient mice to deal with an acute glucose load was assessed by GTT. Although at time 0, young LPL-deficient mice had significantly higher fasting glucose, the glucose excursion curve at the other four data points did not display any significant difference between the two groups (Figure 2a). Indicated by the up-shift of the curve, overt IGT was observed in old LPL-deficient mice, with three data points reaching a statistically significant difference (Figure 2b). Relatively high fasting insulin concentration and insulin resistance as noted above might be a possible explanation for this glucose intolerance.

Early insulin secretion (first 30 min) is important for the rapid and efficient suppression of endogenous glucose production after a meal.[19] To assess early insulin secretion, especially first-phase insulin response (within 10 min), we applied standard intraperitoneal GSIS tests to both young and old mice. As shown in Figures 3a and b, both young and old LPL-deficient mice displayed a significantly reduced insulin peak level at the 5 min time point, even though the extent of response to intraperitoneal glucose injection (as measured by % relative values) was much higher in young than in old mice.

To investigate the mechanism of the impaired insulin response to glucose in severe HTG mice, we examined insulin response to L-arginine in the presence of glucose. Arginine is one of the most important non-glucose stimuli and stimulates insulin secretion at a different step of the cascade. Arginine augmentation of GSIS tests showed a similar decreased first-phase insulin response in LPL-deficient mice compared with the WT group (Figures 3c and d). The impairment in early insulin response in combination with hepatic insulin resistance may result in the excessive early rise of plasma glucose in GTT.

ITT in severe HTG mice

ITT were subsequently used to determine insulin sensitivity and/or insulin resistance, involving an infusion of insulin with subsequent measurements of glucose. The capacity of young LPL-deficient mice to regulate blood glucose concentrations after a bolus intraperitoneal injection of insulin was detected to be slightly higher compared with the WT group (Figure 4a), with one data point reaching significant value ($P < 0.05$). Old severe HTG mice displayed a clear decrease in insulin sensitivity; 15 min after receiving the insulin load the glucose curve shifted downwards significantly (Figure 4b), suggesting systematic insulin resistance in old severe HTG mice.

Pancreas histology in severe HTG mice

Using H–E staining of pancreatic tissue from 12-month-old mice, we observed enlarged islets in LPL-deficient mice, as

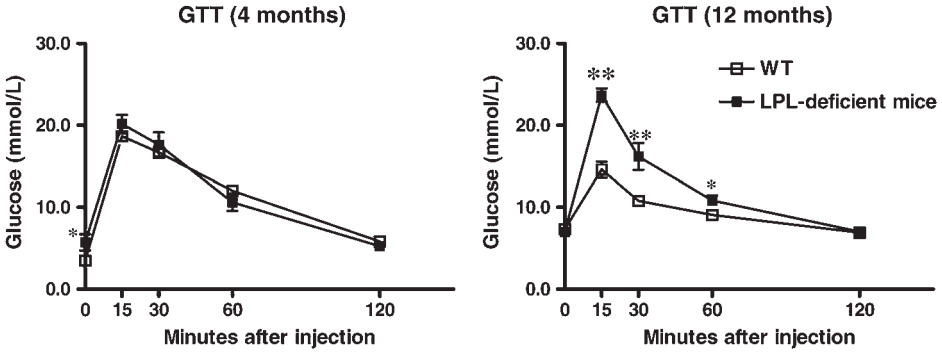

Figure 2 Glucose tolerance tests (GTT) in 4- and 12-month-old lipoprotein lipase (LPL)-deficient mice. Mice were given a bolus intraperitoneal injection of 2 g/kg glucose, and blood glucose was subsequently measured at the indicated time points. $^{*}P < 0.05$, $^{**}P < 0.01$, LPL-deficient mice versus control. $N = 6$ per group. WT, wild type

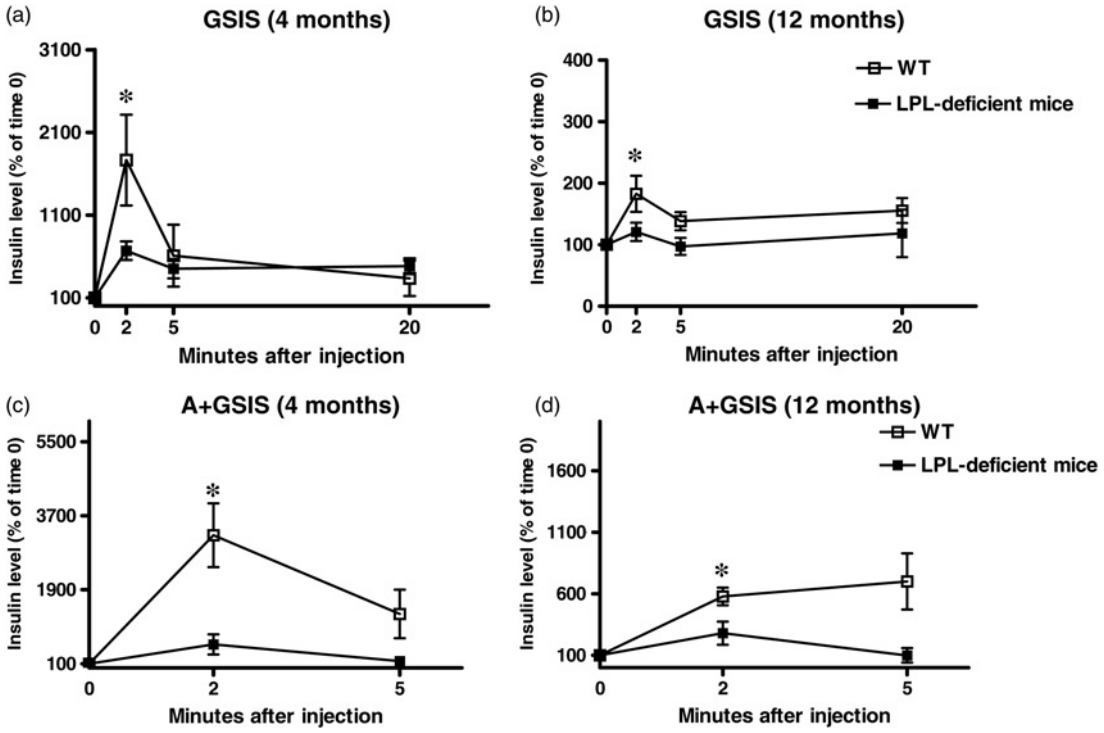

Figure 3 Glucose-stimulated insulin secretion (GSIS) and arginine augmentation of glucose-stimulated insulin secretion (A + GSIS) experiments in 4- and 12-month-old lipoprotein lipase (LPL)-deficient mice. Mice were given a bolus intraperitoneal injection of glucose with or without L-arginine, and blood insulin was measured at the indicated time points. All data compared with basal level as 100%. $^{*}P < 0.05$, LPL-deficient mice versus control. $N = 6$ per group. WT, wild type

shown in Figure 5. The sizes of the islets in LPL-deficient mice were larger than in WT mice ($21{,}991.2 \pm 12{,}093.6$ versus $10{,}339.0 \pm 4223.1$ relative units, $P < 0.05$). No other obvious morphological differences were found in pancreas histological samples between LPL-deficient and WT mice, as viewed by two independent observers with pathological experience.

Discussion

Our study on LPL-deficient mice uncovered a critical influence of severe HTG on glucose homeostasis: age-related glucose intolerance and decreased first-phase insulin secretion by glucose- and/or arginine-stimulation. In the duration of 12 months, glucose metabolism progressively deteriorated from elevated fasting glucose into IGT, accompanied by age-related hyperinsulinemia, decreased insulin sensitivity, enlarged pancreas islets and reduced body weight. Insulin resistance became evident in old LPL-deficient mice.

Recently, Lin *et al.*[7] reported IGT and hyperinsulinemia in 80 non-diabetic severe HTG patients with microalbuminuria, providing clinical evidence of the impact of HTG on glucose metabolism. Additionally, another investigation also showed significant correlation between HTG and type 2 diabetes in LPL Asn291Ser variant carriers in a meta-analysis including 19,246 Caucasians.[20] However, no description about insulin secretion, especially first-phase insulin secretion, one of the earliest signs of diabetes mellitus, was reported in these clinical studies.

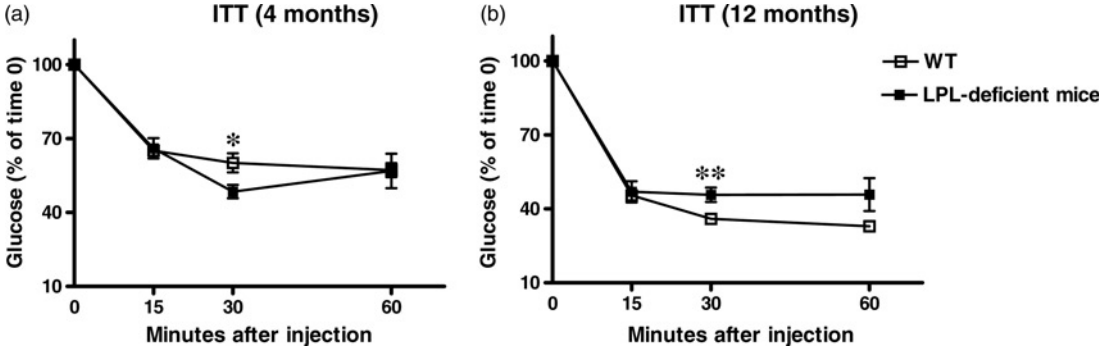

Figure 4 Insulin tolerance tests (ITT) in lipoprotein lipase (LPL)-deficient mice. Mice were given a bolus intraperitoneal injection of 0.75 U/kg insulin, and blood glucose was subsequently measured at the indicated time points. All data compared with basal level as 100%. $^{*}P < 0.05$, $^{**}P < 0.01$, LPL-deficient mice versus control. $N = 6$ per group. WT, wild type

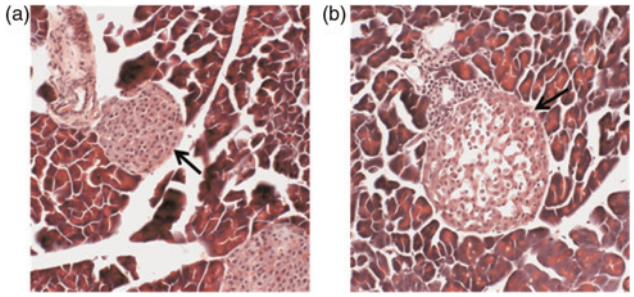

Figure 5 Hematoxylin–eosin staining of pancreas in lipoprotein lipase -deficient mice (b) and control mice (a) of 12 months of age at ×20 magnification and superimposed. Arrowheads indicate pancreas islets. (A color version of this figure is available in the online journal)

Several studies using different HTG animal models have suggested an association between HTG and insulin resistance. Mild expression of human apolipoproteinC1 in ob/ob mice led to severe hyperglycemia and hyperinsulinemia,[21] whereas high expression of apoC1 on ob/ob background was reported by the same authors to be protective from insulin resistance and obesity due to decreased adipose tissue and body weight.[22] HTG transgenic mice of human apoC3, a strong and natural inhibitor of LPL, were shown to be neither insulin resistant nor hyperinsulinemic.[23] Pappan *et al.* reported that β-cell-specific LPL-knockout mice exhibited IGT and a normal lipid profile as well as unchanged fasting insulin concentration.[24] Again, no information about insulin secretion *in vivo* was mentioned in these studies.

With regard to the underlying mechanism governing how severe HTG leads to abnormal glucose metabolism and insulin-resistant status, high FFAs and TG are possibly the predominant factors, for which our previously relevant investigations may provide some further evidence: (1) LPL-deficient mice exhibited elevated plasma FFA concentrations in aged mice.[12] Accumulated FFA is believed to be responsible for skeletal muscle insulin resistance,[25] and for inhibiting glucose oxidation and stimulating gluconeogenesis in the liver, thereby contributing to the inappropriate glucose production;[26] more importantly, chronic exposure of the β-cell to elevated FFA concentrations can cause damage to its function.[27] (2) Massive accumulation of TG-rich CMs in the plasma of these mice resulted in severe oxidative modification of lipid moieties in CMs, as shown by elevated plasma MDA concentration.[28] Furthermore, electron paramagnetic resonance analysis revealed that aged LPL-deficient mice had a striking eight-fold increase in oxidative susceptibility in CMs compared with young mice. (3) CMs from the aged LPL-deficient mice had a stronger effect on upregulation of vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1, markers of enhanced endothelial activation, and increased adherence of human THP-1 mononuclear cells to endothelial cells than CMs from younger mice.[29] Such elevated adhesion molecules and impaired endothelial function are demonstrated to be associated with insulin resistance, possibly further contributing to impaired glucose metabolism.[30]

Thus, chronic HTG and other related factors such as FFAs, inflammatory molecules, insulin resistance and hyperglycemia may interact with one another. At young stage by compensation, LPL-deficient mice displayed normal glucose metabolism or even enhanced insulin sensitivity despite high FFA and TG concentrations, but first-phase insulin secretion decreased as an early sign of β-cell dysfunction. In aged mice, in addition to decreased first-phase insulin secretion they also displayed IGT. In aged mice insulin resistance occurred because of elevated FFA concentrations, impaired endothelial function and severe oxidative modification in CMs.

Conclusions

Using the LPL-deficient mouse model, we provide *in vivo* evidence demonstrating the impact of severe HTG on glucose metabolism and insulin secretion. Although the exact mechanisms for this relationship are yet to be elucidated, the clinical significance of our findings may benefit severe HTG patients resulting from LPL deficiency by the proper management of TG at an early stage of the disorder, thereby reducing the risk of type 2 diabetes in this potentially susceptible patient group.

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