Decreased triglyceride-rich lipoproteins in transgenic skinny mice overexpressing leptin

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Matsuoka, Naoki, Yoshihiro Ogawa, Hiroaki Masuzaki, Ken Ebihara, Megumi Aizawa-Abe, Noriko Satoh, Eiichiro Ishikawa, Yukio Fujisawa, Atsushi Kosaki, Kazunori Yamada, Hideshi Kuzuya, and Kazuwa Nakao. Decreased triglyceride-rich lipoproteins in transgenic skinny mice overexpressing leptin. Am J Physiol Endocrinol Metab 280: E334–E339, 2001.—Leptin is an adipocyte-derived circulating satiety factor with a variety of biological effects. Evidence has accumulated suggesting that leptin may modulate glucose and lipid metabolism. In the present study, we examined lipid metabolism in transgenic skinny mice with elevated plasma leptin concentrations. The plasma concentrations of triglycerides and free fatty acids in transgenic skinny mice were 71.5 (P < 0.01) and 89.1% (P < 0.05) of those in their nontransgenic littermates, respectively. Separation of plasma into lipoprotein classes by ultracentrifugation revealed that very low density lipoprotein-triglyceride concentrations were markedly reduced in transgenic skinny mice relative to the controls. The clearance of triglycerides estimated by a fat-loading test was enhanced in transgenic skinny mice; the triglyceride concentration in transgenic skinny mice 3 h after fat loading was 39.7% (P < 0.05) of that of their nontransgenic littermates. Postheparin plasma lipoprotein lipase activity increased 1.4-fold (P < 0.05) in transgenic skinny mice. Our data demonstrated a significant reduction in plasma triglyceride concentrations, accompanied by increased lipoprotein lipase activity in transgenic skinny mice overexpressing leptin, suggesting that leptin plays a role in long-term triglyceride metabolism.

OBSERVATIONS RESULTS FROM COMPLEX INTERACTIONS AMONG MULTIPLE GENETIC AND ENVIRONMENTAL FACTORS AND IS OFTEN ASSOCIATED WITH METABOLIC DISORDERS SUCH AS DIABETES AND DYSLIPIDEMIA (29). Leptin, a satiety factor that is secreted specifically from the adipose tissue, may signal the presence of excessive energy stores to some nuclei in the brain, which can respond powerfully by reducing food intake and increasing energy expenditure to prevent obesity (5, 8, 19, 22, 35).

Evidence has accumulated suggesting that leptin plays roles in glucose and lipid metabolism (4, 14, 17, 26–28, 32). Leptin-deficient ob/ob mice and leptin receptor-deficient db/db mice developed marked hypercholesterolemia and hypertriglyceridemia (13). Furthermore, recent studies have revealed an acute and substantial fall in plasma lipid concentrations in ob/ob mice but not in db/db mice when treated with even low doses of leptin that do not affect food intake or body weight (10, 33). These findings suggest that leptin can regulate lipid metabolism independently of changes in adiposity. However, the impact of chronic hyperleptinemia on lipid metabolism has not been clarified.

Dietary lipids are taken up in the small intestine and drained as chylomicrons and free fatty acids (FFAs) into the liver, where they are reassembled into very low density lipoprotein (VLDL) particles, whereupon they are released into the blood circulation. With apoB (apoB), triglycerides are assembled into chylomicron and VLDL with the aid of the microsomal triglyceride transfer protein (MTP) in the intestine and liver, respectively (11, 34). The circulating triglyceride-rich lipoproteins are known to be hydrolyzed by lipoprotein lipase (LPL) (23, 24). LPL is secreted from the parenchymal cells of various extrahepatic tissues, mainly skeletal muscle and adipose tissue, and is anchored to the luminal surface of the capillary endothelium. On the other hand, intermediate- and high-density lipoproteins (IDL and HDL) are hydrolyzed by hepatic triglyceride lipase (HTGL). HTGL is secreted exclusively from the liver and is anchored via heparan sulfate proteoglycans to the surface of hepatocytes and liver sinusoid endothelial cells.

To assess the long-term biological effects of leptin in vivo, we recently bred transgenic skinny mice overexpressing leptin under the control of a liver-specific promoter and demonstrated that chronic hyperleptin-
mice results in the complete disappearance of brown and white adipose tissue (17). The mice exhibited increased glucose tolerance and insulin sensitivity accompanied by the activation of insulin signaling in the skeletal muscle and liver. Plasma insulin concentrations in transgenic mice are one-third those in their nontransgenic littermates, although there is no significant difference in plasma glucose concentrations between the two genotypes. Thus transgenic skinny mice provide a useful model to investigate the metabolic effects of leptin on a long-term basis (13, 17).

The present study was designed to elucidate the effects of chronic hyperleptinemia on lipid metabolism, in particular triglyceride metabolism, using transgenic skinny mice. We analyzed their plasma lipid profiles, plasma triglyceride clearance, and plasma lipase activities; we also examined the expression of mRNAs for key enzymes in triglyceride metabolism.

MATERIALS AND METHODS

Materials and transgenic mice. [α-32P]dCTP and glycerol tri[9,10(n)-3H]oleate were purchased from New England Nuclear Life Science Products, Tokyo, Japan. Enzymes and other chemicals were of reagent grade. Generation of transgenic skinny mice overexpressing leptin and some of their characteristic phenotypes have been reported elsewhere (1, 14, 17). In the present study, we analyzed male transgenic skinny mice with ~30 copies of the heterozygous transgene and their nontransgenic littermates. They were housed in a temperature-, humidity-, and light-controlled room (12:12-h light-dark cycle) with free access to a standard chow diet (CE-2; 352 kcal/100 g, Japan CLEA, Tokyo, Japan). After measurement of the food intake of transgenic mice, a pair-fed control group was prepared by limiting the food intake of some nontransgenic mice for 3 wk. At 14 wk of age, the transgenic skinny mice had gained less weight than their nontransgenic littermates, with no apparent brown or white adipose tissue as reported (17). All experimental protocols were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research.

Plasma lipid profiles and hepatic triglyceride content. Blood was drawn from the retroorbital sinus of 14-wk-old transgenic mice and their nontransgenic littermates under ethyl ether anesthesia. Plasma concentrations of triglycerides, FFAs, and cholesterol were determined enzymatically (Triglyceride G-test Wako, NEFA C-test Wako, and Cholesterol E-test Wako, respectively; Wako Pure Chemicals, Osaka, Japan). Blood samples were collected from the inferior vena cava of 12-wk-old transgenic mice and their nontransgenic littermates under diethyl ether anesthesia after a 5-h fast with or without a 2-h refeeding. Mice were fasted for only 5 h, because in an earlier study some transgenic skinny mice did not survive during a longer period of fasting (unpublished data). Pooled plasma (600 μl) was separated by potassium bromide continuous density gradient ultracentrifugation as described previously (30) with slight modifications (100,000 rpm for 25 min). The triglycerides and cholesterol of each fraction were measured and corrected for the difference in recovery. Two lipopropak LX columns (TOSOH, Tokyo, Japan) were perfused at 0.6 ml/min, 25°C with LP-1 (TOSOH). Determiner-L TC (Kyowa Medix, Tokyo, Japan) was used as a reaction agent at 0.3 ml/min, 45°C, and the elute was monitored at 550 nm. A high-performance liquid chromatography system (Shimadzu LC10, Shimadzu, Kyoto, Japan) gave four major, distinct elution peaks corresponding to chylomicrons, VLDL, LDL, and HDL. After blood sampling, the liver was removed and weighed. Hepatic lipids were extracted with hexane-isopropyl alcohol (3:2) from a piece of frozen liver. After evaporation of organic solvent, the lipids were resuspended in ethanol, and the triglyceride content was measured with an enzymatic kit (31).

Fat-loading test and plasma lipase activity. A fat-loading test was performed using 14-wk-old ad libitum-fed transgenic mice and their ad libitum- and pair-fed nontransgenic

![Fig. 1. Lipoprotein profiles analyzed by ultracentrifugation.](http://ajpendo.physiology.org/)
littermates (n = 4). During fasting, when mice were given free access to water, 400 μl of olive oil were administered per os to all groups. With mice under anesthesia, an aliquot of blood was sampled serially before and 1, 2, 3, and 5 h after fat loading, and plasma triglyceride concentrations were measured.

To assess the plasma lipase activities after fasting, postheparin plasma was sampled from ad libitum-fed mice of both genotypes (n = 5) 15 min after a bolus injection of heparin (100 U/kg) into the femoral vein. Heparin-releasable LPL activity was assayed using the method described previously (24).

**Northern blot analysis.** After mice were fasted, total RNAs were extracted from the heart, gastrocnemius muscle, and liver under anesthesia. Northern blot analysis was performed as described (15) using cDNA fragments of mouse leptin, LPL (11), and MTP (16) as probes. The mRNA abundance was quantitated with a BAS-2500 Computer Bioimaging Analyzer System (Fuji Film, Tokyo, Japan).

**Statistical analysis.** Values are expressed throughout as means ± SE and were analyzed using Mann-Whitney’s nonparametric U-test and the Bonferroni-Dunn test with a statistical software package (Statview J.4.02; Abacus Concepts, Berkeley, CA). A P value < 0.05 was considered significant.

**RESULTS**

**Plasma lipid profiles and hepatic lipid contents.** The plasma triglyceride and FFA concentrations in transgenic skinny mice were 71.5% (0.87 ± 0.02 vs. 1.21 ± 0.09 mM, P < 0.01) and 89.1% (1.23 ± 0.04 vs. 1.38 ± 0.01 mM, P < 0.05), respectively, of those in their ad libitum-fed nontransgenic littermates. However, significant difference was not observed in plasma cholesterol concentrations between the genotypes (2.01 ± 0.13 vs. 2.05 ± 0.05 mM). Analysis of plasma lipoprotein fractions separated by ultracentrifugation revealed a low amount of triglycerides in VLDL fractions from transgenic skinny mice relative to their nontransgenic littermates (Fig. 1). The amount of HDL cholesterol was lower in transgenic skinny mice than in their nontransgenic littermates (about two-thirds of that in nontransgenic littermates). HPLC identified four distinct elution peaks corresponding to chylomicron, VLDL, LDL, and HDL (Fig. 2). Two hours after refeeding, the amounts of VLDL, LDL, and HDL were lower, whereas the number of chylomicrons was higher in transgenic skinny mice than in their nontransgenic littermates. Similarly, after a 5-h fast, the amounts of VLDL and HDL were lower, whereas the amount of LDL was higher in transgenic skinny mice. The chylomicron peak was barely detectable in transgenic skinny mice during fasting, but a peak of intermediate size between chylomicrons and VLDL increased in transgenic skinny mice. Liver weight was lower in transgenic skinny mice than in their nontransgenic littermates (Fig. 3A). Hepatic triglyceride content was significantly lower in transgenic skinny mice than in their nontransgenic littermates (Fig. 3B).

**Fat-loading test and plasma lipase activities.** To investigate the clearance of triglycerides, exogenous triglycerides were loaded as dietary fat to transgenic skinny mice and their nontransgenic littermates (Fig. 4). Throughout the fat-loading test, plasma triglyceride concentrations were lower in the transgenic skinny mice than in their ad libitum-fed nontransgenic littermates. There was a significant difference in plasma triglyceride concentrations between transgenic mice and their ad libitum-fed nontransgenic littermates 2 and 3 h after fat loading. A significant difference in plasma triglyceride concentrations between transgenic mice and their pair-fed nontransgenic littermates was observed 2 h after fat loading.

We also determined LPL activities in postheparin plasma. In transgenic skinny mice, LPL activity was 1.4-fold higher than that in their nontransgenic littermates (Fig. 5).

**Northern blot analysis.** Northern blot analysis revealed no significant changes in LPL mRNA level in
cardiac and skeletal muscles from transgenic skinny mice compared with those of their nontransgenic littermates (Fig. 6). No appreciable difference in MTP mRNA levels in the liver was noted between genotypes.

**DISCUSSION**

The present study demonstrated a decrease in plasma triglyceride concentrations, especially the VLDL-triglyceride fraction, in transgenic skinny mice relative to their nontransgenic littermates. The amount of HDL cholesterol was lower in transgenic skinny mice than in their nontransgenic littermates. This is consistent with a recent report that leptin can acutely decrease plasma HDL concentrations in leptin-deficient ob/ob mice (26). Silver et al. (27) also reported that a major defect in HDL particle uptake in ob/ob hepatocytes causes decreased recycling, degradation, and selective lipid uptake, which were reversed by low-dose leptin administration. These two reports indicated that leptin plays important roles in lipid metabolism.

Triglyceride contents in the liver were significantly decreased in transgenic skinny mice relative to those in their nontransgenic littermates. These observations suggest that leptin can chronically increase triglyceride metabolism. Transgenic skinny mice eat less than their nontransgenic littermates under ad libitum feeding conditions. The data from the pair-fed control group suggests that reduced uptake of dietary lipids in the intestine may partly contribute to decreased plasma triglyceride concentrations. Two hours after fat loading, there was a significant difference between transgenic mice and pair-fed nontransgenic mice. Throughout the time course of the fat-loading test, plasma triglyceride concentrations were lower in

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**Fig. 3.** Liver weight (A) and hepatic triglyceride content (B). After blood was drawn, the livers of the TGM (solid bars) and ad libitum-fed nonTGM (open bars) were extracted and weighed (liver wt (g)/body wt (g); A), and the triglyceride contents by liver weight (μmol/g tissue wt; B) were measured. *P < 0.05 vs. non-TGM.

**Fig. 4.** Triglyceride levels in a fat-loading test. Changes in plasma triglyceride concentrations in 14-wk-old TGM (●), pair-fed nonTGM (□), and ad libitum-fed nonTGM (■) during a fat-loading test. A total of 400 μl of olive oil was orally loaded to each mouse. Before and 1, 2, 3, and 5 h after administration, triglyceride concentrations were measured. *P < 0.05, **P < 0.01 vs. ad libitum-fed nonTGM at the same time points. †P < 0.05 vs. pair-fed nonTGM at the same time points.

**Fig. 5.** Postheparin plasma lipoprotein lipase (LPL) activity. Postheparin plasma LPL activity in 15-wk-old TGM (solid bar) and ad libitum-fed nonTGM (open bar) was measured. After fasting, postheparin plasma was sampled from mice (n = 5) 15 min after a bolus injection of heparin (100 U/kg) into the femoral vein. Lipase activity was analyzed using glycerol tri[9,10(α)]-H]oleate. Data are from 3 independent assays. *P < 0.05 vs. nonTGM.
transgenic skinny mice than those in their ad libitum-fed nontransgenic littermates. Furthermore, Shimomura et al. (25) recently demonstrated that exogenous leptin administration could reduce plasma and hepatic triglycerides without affecting food intake in mice with congenital lipodystrophy. Taken together, it is reasonable to speculate that chronic hyperleptinemia can increase triglyceride metabolism independently of its satiety effect in transgenic skinny mice.

On the other hand, no appreciable changes in MTP mRNA expression were noted in livers between genotypes, suggesting that VLDL secretion is not increased in the liver in the transgenic skinny mice. These observations suggest that decreased plasma triglyceride concentrations are partly due to increased clearance of triglycerides from the blood circulation in transgenic skinny mice.

In the present study, postheparin plasma LPL activity increased in transgenic skinny mice relative to their nontransgenic littermates, suggesting that hydrolysis or turnover of triglyceride-rich lipoproteins is accelerated in transgenic skinny mice. The mechanism by which plasma LPL activity is increased in transgenic skinny mice is not clear at present. The LPL activity is regulated at multiple steps via transcriptional, translational, and/or posttranslational mechanisms (2, 3, 9, 23, 24). A previous study showed that leptin can up-regulate LPL mRNA expression in adipose tissue and skeletal muscle (21). In this regard, transgenic skinny mice are apparently deficient in white adipose tissue (14, 17), one of the major sources of LPL. Furthermore, between genotypes, there are no appreciable differences in LPL mRNA levels in cardiac and skeletal muscles, which are other major production sites of LPL. Thus chronic hyperleptinemia could increase plasma LPL activity derived from cardiac and skeletal muscles via posttranscriptional mechanisms. We also reported increased insulin sensitivity in the skeletal muscle and liver from transgenic skinny mice (14, 17). Because LPL activity is regulated positively by insulin action in vivo (18), it is conceivable that chronic hyperleptinemia can activate LPL activity through increased insulin sensitivity in transgenic skinny mice. Recently, Wang et al. (32) have shown that, in normal adipocytes, leptin directly stimulates a novel form of lipolysis in which glycerol is released without a proportional release of FFA. By use of the conventional method, in which free glycerol is also counted as esterified glycerol, higher plasma triglyceride concentration was observed in leptin transgenic skinny mice (data not shown).

Clinically, it would be interesting to discover whether leptin can be used for the treatment of obesity-related diabetes-dyslipidemia syndrome. Overnutrition stimulates synthesis and secretion of leptin in the adipocytes, which should represent one of the defense mechanisms against the development of obesity and obesity-related metabolic disorders. In obese subjects, however, plasma leptin concentrations are markedly elevated, suggesting a state of “leptin resistance” (6, 13). There are papers reporting the facts in humans contradictory to our results. Rainwater et al. (20) reported that HDL-triglyceride and the proportion of apoA-I on HDL particles larger than HDL20 (large HDLapoA-I) were independently correlated with serum leptin concentration in humans. Couillard et al. (7) reported that significant associations between plasma leptin and lipoprotein concentrations were eliminated by statistical adjustment for body fat mass in both men and women. It is very difficult to distinguish the “effects of leptin” from the “facts observed in obese, or hyperleptinemic, subjects with leptin-resistance.” Furthermore, the difference in lipid metabolism between rodents and humans makes it more complicated. Using transgenic skinny mice with the agouti allele, we recently demonstrated that, during caloric restriction, sustained hyperleptinemia can accelerate the recovery of insulin resistance in the obesity-diabetes syndrome and postulated the potential usefulness of exogenous leptin administration in combination with caloric restriction for the treatment of obesity-related diabetes (14).

In conclusion, we have demonstrated reduced triglycerides due to increased clearance from the circulation, accompanied by increased LPL activity, in transgenic skinny mice overexpressing leptin. Our results also suggest the potential usefulness of leptin as a novel therapeutic strategy for obesity-associated diabetes-dyslipidemia syndrome in combination with, for instance, caloric restriction, when leptin resistance is improved.

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