

Decreased lipoprotein clearance is responsible for increased cholesterol in streptozotocin treated LDL receptor knockout mice

Ira J. Goldberg M.D., Yunying Hu B.S., Hye-Lim Noh, Ph.D., Justin Wei B.S., Lesley Ann Huggins B.S., Marnie G. Rackmill B.A., Hiroko Hamai M.D., Brendan N. Reid B.A., William S. Blaner Ph.D., Li-Shin Huang Ph.D.

Divisions of Preventive Medicine and Nutrition, and Cardiology; Columbia University, New York, NY

Running Title: ApoB is increased in diabetic mice

Corresponding Author:

Ira J. Goldberg, M.D.
Department of Medicine
Columbia University
630 West 168th Street
New York, NY 10032
ijg3@columbia.edu

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ABSTRACT

Objective: Patients with diabetes often have dyslipidemia and increased postprandial lipidemia. Induction of diabetes in LDL receptor (*Ldlr*^{-/-}) knockout mice also leads to marked dyslipidemia. The reasons for this are unclear.

Research Design and Methods: We placed *Ldlr*^{-/-} and heterozygous LDL receptor knockout (*Ldlr*^{+/-}) mice on a high cholesterol (0.15%) diet, induced diabetes with streptozotocin (STZ), and assessed reasons for differences in plasma cholesterol.

Results: Diabetic *Ldlr*^{-/-} mice had plasma cholesterol levels more than double those of non-diabetic controls. FPLC and ultracentrifugation showed an increase in both VLDL and LDL. Plasma VLDL became more cholesterol-enriched and both VLDL and LDL had a greater content of apoE. In LDL the ratio of apoB48 to apoB100 was increased. ApoB production, assessed using [³⁵S]methionine labeling in Triton WR1339-treated mice, was not increased in fasting diabetic mice. Similarly, postprandial lipoprotein production was not increased. Reduction of cholesterol in the diet to normalize the amount of cholesterol intake by the control and diabetic animals reduced plasma cholesterol levels in diabetic mice, but plasma cholesterol was still markedly elevated compared to non-diabetic controls. LDL from diabetic mice was cleared from the plasma and trapped more rapidly by livers of control mice. STZ-treatment reduced liver expression of the proteoglycan sulfation enzyme, *Ndst1*, an effect that was reproduced in cultured hepatocytes by high glucose-containing medium.

Conclusion: Diabetic cholesterol-fed mice developed hyperlipidemia due to a non-LDL receptor defect in clearance of circulating apoB-containing lipoproteins.

Diabetes leads to a marked increase in atherosclerosis manifesting as coronary artery disease, stroke and peripheral vascular disease. Because the presence of diabetes leads to such a marked increase in disease (1), recent guidelines consider the presence of diabetes as equivalent to the presence of known atherosclerosis (2; 3). However, the reasons that diabetes leads to greater atherosclerosis are unknown (4; 5) and there is a deficiency of animal models to study this problem. In recognition of this, the NIH instituted a program to develop models of diabetes-induced complications (<http://www.amdcc.org/>).

A number of studies have been performed to assess the effects of diabetes on atherosclerosis in mice. ApoE knockout (*ApoE*^{-/-}) mice (6; 7) and LDL receptor knockout (*Ldlr*^{-/-}) mice (8-10) often develop marked hyperlipidemia with diabetes. In the case of the *ApoE*^{-/-} mice, circulating lipoproteins are deficient in apoE, the ligand needed for the interaction of remnant lipoproteins with both the LDL receptor and the LDL receptor related protein (LRP). Hepatic removal of these remnant lipoproteins might require proteoglycan trapping in the liver; diabetic livers have a defect in proteoglycan production (7). *Ldlr*^{-/-} mice have apoB-containing lipoproteins (VLDL and LDL) containing apoE that should be capable of interacting with LRP as well as proteoglycans.

Humans with diabetes do not, in general, have increased circulating levels of fasting lipoproteins, however, their ability to clear postprandial lipoproteins is uniformly impaired (11). This is either due to defective plasma triglyceride lipolysis or to impaired clearance of whole lipoproteins. Mice with diabetes and LDL receptor defects often develop severe hyperlipidemia with streptozotocin (STZ)-induced diabetes (8; 10),

while similarly treated diabetic mice without loss of this receptor have only minor lipoprotein changes (12). This suggests that the defective pathways required to clear lipoproteins in the diabetic mice are exclusive of the LDL receptor. To determine the cause of the lipoprotein metabolic defect in *Ldlr*^{-/-} mice, we studied lipoprotein profiles, liver and intestinal lipoprotein production, and acute plasma lipoprotein turnover in control and diabetic *Ldlr*^{-/-} mice.

METHODS

Mice, diets and induction of diabetes. *Ldlr*^{-/-} mice on the C57BL/6 background (B6.129S7-LDLr^{tm1Her}, stock No.002207) were purchased from Jackson Laboratory. PCR genotyping for loss of the *Ldlr* was as described (13). Mice were maintained in a temperature-controlled (25°C) facility with a 12-hour light/dark cycle and given free access to food and water, except when fasting blood specimens were obtained. Mice were fed either laboratory rodent chow (PMI Nutrition International Inc.), semisynthetic modified AIN76 diet containing 0.15 or 0.075% cholesterol (14) (high cholesterol diet), or a diet containing cholesterol and cholic acid (15). The Institutional Animal Care and Use Committee of Columbia University approved all animal protocols.

Mice were made diabetic by administering 50 mg/kg body weight STZ intraperitoneally for 5 days. Only male mice were used for these studies. Four weeks after STZ administration diabetic and non-diabetic mice with LDL receptor deficiency were kept on diets for 4 weeks.

Blood sampling. Plasma samples were obtained from 6-hour fasted mice. Glucose was measured directly from the tail tip with a glucometer; total cholesterol and triglycerides were measured enzymatically using commercial kits (Wako Chemicals).

Lipoprotein analysis. Lipoproteins, VLDL ($d < 1.006$ g/ml), IDL + LDL ($d = 1.006$ – 1.063 g/ml), and HDL ($d = 1.063$ – 1.21 g/ml), were separated by sequential density ultracentrifugation of plasma in a TLA100 rotor (12). Sixty μ l sample volumes were utilized.

Pooled plasma (from between 3 and 5 animals, 200 μ l total volume) was chromatographed using two Superose 6 columns in series (16). Fifty 0.5 ml fractions were collected. Cholesterol and triglyceride levels of FPLC fractions were measured using enzymatic reagents (as in plasma analysis) in colorimetric assays and measured on the SpectraMax 250.

Tissue collection. At sacrifice hearts were perfused with 10 ml cold PBS. Adequacy of the perfusion was evident from blanching of the livers. Tissues were removed and immediately frozen in liquid nitrogen.

Gene expression. Total RNA (10 μ g) was isolated from livers using Trizol reagent. Quantitative real-time PCR was performed with SYBR Green PCR Core Reagents. Incorporation of the SYBR green dye into the PCR products was monitored in real time with an Mx3000 sequence detection system. Primer pairs were designed and used to assess mRNA levels of LRP, scavenger receptor B-I (SR-BI) and heparan sulfate *N*-deacetylase/*N*-sulfotransferase-1 (Ndst1). Samples were normalized against β -actin.

Western blot. SDS-PAGE was performed on a 12% acrylamide gel using NOVEX XCell II electrophoresis apparatus. Proteins were stained with Coomassie blue. For immunoblot, after electrophoresis proteins were transferred onto a nitrocellulose membrane in Tris-glycine buffer, pH 8.3 containing 20% (v/v) methanol at 25 mV for 90 min. Non-specific binding of the membrane was blocked with 5% non-fat dry milk. The membrane was incubated in PBS containing polyclonal antibodies against SR-BI and LRP at 4°C overnight and then further

incubated in PBS containing anti-goat IgG coupled with horseradish peroxidase. The blots were developed with enhanced chemiluminescence (ECL) reagent, exposed to film, and scanned using a Molecular Dynamics 300A laser densitometer.

ApoB and apoE analysis. VLDL and LDL were isolated by ultracentrifugation, as described previously (17). For apoE, recovered lipoproteins from equal volumes of plasma were analyzed on a 4-20% gradient gels. The apoE bands were identified by molecular weight. Lipoproteins obtained from apoE knockout mice, were used as a negative control. For apoB, samples from 4 control *Ldlr*^{-/-} and 5 STZ-treated *Ldlr*^{-/-} mice were separated on self-made 4% SDS-PAGE.

The relative amounts of apoB100 and apoB48 were determined by SDS-PAGE followed by Western Blot analysis. After apoB isoforms were resolved, they were transferred to nitrocellulose membranes, incubated with an anti-apoB antibody and secondary antibody, and developed as described above.

Triglyceride and retinyl ester uptake. Mice were fasted for 6 hours and then received 50 μ l of a cocktail containing 1 μ Ci [³H]retinol via nasogastric gavage (18). Blood was obtained from retroorbital bleeds at 3, 6 and 10 hour. Plasma triglyceride and ³H counts were measured. To determine the retinyl label within chylomicrons, and not that associated with retinol-binding protein, density <1.006 mg/dL lipoproteins were isolated. Plasma and tissue ³H counts were measured using 3.5 ml of Hydroflor liquid scintillation cocktail.

ApoB production. ApoB production was determined in fasted and fed mice with two different protocols. To measure hepatic apoB production, mice were fasted for 4 hours and then injected with a mixture of [³⁵S]Promix (0.2 mCi/mouse) and Triton WR-1339 (500 mg/kg) to block lipolysis as described (17). Blood was taken pre-injection and at 60 and 120 minutes post-injection. Plasma was

isolated and radioactivity of newly synthesized apoB100 and apoB48 was assessed by SDS-PAGE. Counts for apoB48 and apoB100 were normalized to total TCA-precipitable counts in plasma.

To more preferentially assess intestinal lipoprotein production, mice received an intraperitoneal injection of the total lipase inhibitor, P-407 (1 g/kg body weight), approximately 2 hours before the experiment began. Two hours later the mice were gavaged with 200 μ l of peanut oil and 200 μ Ci of [³⁵S]Promix. Four hours after the P-407, blood and tissues were obtained. VLDL were isolated from plasma by ultracentrifugation, apoB proteins separated by SDS-PAGE and the radioactivity of apoB100 and apoB48 counted and normalized to total TCA-precipitable counts in plasma.

Lipoprotein decay and liver trapping. LDL (d=1.019-1.063 g/ml) and VLDL (d<1.006 g/ml) from control and STZ-treated mice were isolated. Lipoproteins from the STZ-treated mice were labeled with ¹²⁵I and those from control *Ldlr*^{-/-} mice were labeled with ¹³¹I using the lactoperoxidase method (19). After a 6-hour fast, control and STZ-treated mice received labeled lipoproteins (~0.5 μ Ci of ¹²⁵I and 0.005 μ Ci of ¹³¹I) by tail vein injection. Blood was taken at 30 seconds and used to recalculate injected dose by estimating total plasma as 4% of body weight. Ten minutes after injection blood was obtained, the animals were perfused with 10 ml of 4°C saline, and organs were harvested and weighed. The radioactivity was determined using a dual isotope counter and spillover corrected.

McArdle cells, culture and gene expression. McArdle rat hepatoma cells were seeded on plates coated with collagen I and grown in DMEM with 10% FBS and 10% horse serum. To examine the effects of glucose, cells were plated in 6-well plates. After overnight incubation, cells were switched to medium with 5 or 25mM glucose for 24h. Cells were

harvested and mRNA analyzed for *Ndst1* gene expression.

STATISTICAL ANALYSIS

Statistical analyses were done by unpaired t-test or ANOVA. All data are expressed as mean \pm SD (?), with a statistically significant difference defined as a value of $p < 0.05$.

RESULTS

Effects of diabetes and diets on plasma lipids and lipoproteins. Mice were made diabetic with STZ and the animals were fed a high cholesterol diet (0.15%) with no added fat. Fat was omitted from the diet because it causes insulin resistance in these mice and might have obscured diabetic effects from insulin deficiency. Approximately 80% of the mice became diabetic, defined as having fasting glucose >250 mg/dL. These animals had average glucose levels of 524 \pm 108 mg/dL, whereas nondiabetic controls had an average glucose level of 151 \pm 15 mg/dL (Table 1). After four weeks on the high cholesterol diet, fasting plasma cholesterol in *Ldlr*^{-/-} mice was twice that of their non-diabetic littermates, 1696 \pm 478 versus 884 \pm 134 mg/dL. Plasma triglyceride levels were not significantly increased. A similar increase in plasma cholesterol levels was also noted in a group of STZ-treated mice fed a cholesterol/cholic acid diet (Table 1).

The importance of the lack of LDL receptor in this response to the diet was assessed by placing a group of heterozygous male LDL receptor deficient mice (*Ldlr*^{+/-}) on the 0.15% cholesterol diet. Despite a lower level of glucose in this group, the *Ldlr*^{+/-} mice also had increased plasma cholesterol levels with STZ-treatment. In contrast, it should be noted that STZ treatment leads to minimal differences in cholesterol levels in wild-type mice (20).

Plasma lipoprotein profiles from the control and STZ-treated *Ldlr*^{-/-} mice were obtained after 4 weeks on the 0.15%

cholesterol diet. Isolation of particles by ultracentrifugation showed that high cholesterol diet-fed STZ-treated *Ldlr*^{-/-} mice had a marked increase in both VLDL and LDL cholesterol, but the changes in TG were not significant. This change in the lipoprotein profile was also found by FPLC analysis (Fig. 1); VLDL and LDL cholesterol, but not TG levels, were increased. In addition, both the VLDL and LDL eluted at a large size, suggesting that the greater cholesterol content was due to the presence of more lipid-rich lipoproteins. HDL were reduced in both the centrifugation and FLPC profiles (Fig. 1A, inset).

ApoB and apoE distribution in isolated lipoprotein fractions: To determine whether STZ-treatment led to marked changes in lipoprotein apoprotein composition, isolated VLDL and LDL from control and diabetic mice fed the cholesterol-containing diet for 4 weeks were studied. Triglyceride-rich lipoproteins and LDL contain either apoB100 or apoB48 as their major protein. ApoE content of these VLDL and LDL was determined by PAGE using lipoproteins isolated by ultracentrifugation. ApoE normally mediates liver uptake of apoB48 lipoproteins. ApoE in both VLDL and LDL was increased and increased in proportion to apoB48 (Fig. 2A). This was consistent with there being either a defect in lipoprotein plasma clearance or increased lipoprotein production.

To determine which type of lipoproteins were increased, VLDL and LDL from control and STZ-treated mice were analyzed for apoB size by gel electrophoresis followed by Western blot analysis. In non-diabetic mice, the LDL was composed primarily of apoB100-containing particles with some apoB48 (Fig. 2B). However, in STZ-treated mice, the distribution was altered; much of the apoB was now apoB48 (Fig. 2B). Although the B100/B48 ratio tended to also decrease in VLDL after STZ treatment, this

change in apoB distribution was not significant.

ApoB production: In humans, apoB48 is synthesized in the intestines, whereas mice also secrete apoB48 from the liver. To determine whether liver production of lipoproteins was responsible for the plasma hyperlipidemia in the diabetic mice, we determined whether STZ-treatment altered apoB production in fasting control and diabetic mice fed the cholesterol-containing diet for 4 weeks. As shown (Fig. 3A-C), the diabetic mice showed no increase in liver production of apoB or triglycerides; actually production of both apoB100 and apoB48 were similar in diabetic and control mice. Thus, the increase in apoB48 lipoproteins was either due to reduced clearance or increased postprandial lipoprotein production, the latter could relate to changes in dietary input (see below).

Similarly, we assessed apoB production with P-407 treated mice that received a gavage of corn oil containing [³⁵S]methionine. Again, STZ-treated mice showed no evidence of increased apoB production and, in fact, appeared to have reduced production of apoB (Figure 3D).

Lipoprotein receptors. In *Ldlr*^{-/-} mice, reduced levels of LRP and/or SR-BI might have decreased plasma clearance of lipoproteins. However, liver LRP and SR-BI mRNA expression was surprisingly increased in the diabetic mice (Fig. 4A). When these proteins were assessed by Western blot analysis (Fig. 4B), there appeared to be no difference in liver content of the two receptors. Thus, reduced lipoprotein clearance via these receptors is unlikely to have led to the hypercholesterolemia in the diabetic mice.

Absorption and metabolism of a standard meal. We determined whether absorption of fats and chylomicron metabolism were altered in the diabetic mice using radioabeled [³H]retinyl ester. By 3 hours, plasma triglyceride levels increased in both groups of

mice from 167 ± 23 to 237 ± 25 mg/dL in the control and 133 ± 24 to 225 ± 45 mg/dL in the STZ-treated group. There was also no difference in the peak or clearance of radiolabeled retinyl ester within the density <1.006 particles, (i.e. chylomicrons and chylomicron remnants)(not shown).

Effects on food intake. Since lipoprotein production under standard conditions was not increased, we assessed whether the greater amount of apoB was associated with greater intake of cholesterol. Food intake in the STZ-treated animals was 5.5 g/mouse per day while in control mice it was 3.0 g/mouse per day; this is a 1.8 fold increase. To determine how much of the diabetes-induced hyperlipidemia was due to diet, STZ-treated mice were given 0.15 and 0.075% cholesterol diets. After 4 weeks, STZ-treated mice on the 0.15% cholesterol diet had plasma cholesterol levels of 2007 ± 844 mg/dL, which was significantly different from the non-diabetic mice which had cholesterol levels of 767 ± 270 mg/dL (Table 2). Diabetic mice on the 0.075% cholesterol had plasma cholesterol levels of 1697 ± 629 mg/dL, levels not significantly different from those in the diabetic mice consuming the 0.15% cholesterol diet. Therefore, compensation for the increased dietary cholesterol did not correct the excess hyperlipidemia in the diabetic mice.

Lipoprotein decay and trapping. To determine the fate of plasma lipoproteins, we assessed whether there was a defect in initial trapping of lipoproteins. To do this, 10 minute plasma decay and liver uptake of LDL and VLDL isolated from control and STZ-treated mice were assessed in control and STZ-treated *Ldlr*^{-/-} mice. As shown in Fig. 5A and B, plasma decay and liver uptake of ¹²⁵I-STZ-LDL was greater in control than diabetic mice. Uptake of LDL into other tissues was not significantly altered; there were no significant differences in the percent uptake of either tracer into heart, skeletal muscle,

adipose, kidney or spleen (data not shown). Thus, livers from control mice cleared LDL from the diabetic mice more rapidly. This suggested STZ-LDL had a greater affinity for liver proteoglycans in control, than diabetic mice.

When labeled STZ-mouse derived VLDL was injected, a similar trend was noted, i.e. reduced liver trapping and increased plasma tracer in diabetic mice, but there was greater variability between mice and the differences did not reach significance, $p=0.12$ (Fig. 5A and B). In contrast, LDL and VLDL isolated from control animals were cleared at the same rate by the diabetic and control mice (not shown). Therefore, a subgroup of particles accumulated in the diabetic mice that were rapidly cleared by the livers of non-diabetic animals.

Liver sulfation enzyme expression. We had previously shown that sulfation of heparan sulfate proteoglycans was defective in STZ-treated mice. As had been reported by others (21), we noted that *Ndst1* mRNA levels were reduced in livers from diabetic mice (Fig. 6A). To determine the mechanism for this, we assessed the effects of glucose concentration on expression on *Ndst1* in cultured hepatocytes. Growing the cells 24 h in 25 mM glucose reduced *Ndst1* mRNA levels (Fig. 6B).

DISCUSSION

Studies in diabetic mice have shown either increased or no change in atherosclerosis (22). Increased atherosclerosis has been associated with, and perhaps caused by, greater hyperlipidemia in studies with *Apoe*^{-/-} (6; 23; 24), human apoB transgenic (16), and *Ldlr*^{-/-} mice (9; 10). In other studies, when minor or no change in circulating lipids was found, atherosclerosis was unaltered despite the development of either insulin resistance or diabetes (12; 25-27). Unlike *Apoe*^{-/-} mice which lack the ligand to allow remnant lipoproteins to interact with both the

LDL receptor and LRP, *Ldlr*^{-/-} mice have circulating VLDL remnants and even LDL (28) that contain apoE. In part for this reason, these two models differ in their lipoprotein response to diet-induced insulin resistance (29). Another diabetic model, leptin deficiency, is associated with marked hyperlipidemia when crossed onto the *Ldlr*^{-/-} background (30). However, leptin deficiency itself alters lipid metabolism; it raises HDL levels and reduces SR-BI expression (31).

In this study, we sought to define the pathophysiological process leading to hyperlipidemia in STZ-treated *Ldlr*^{-/-} mice. This process has complicated studies of atherosclerosis that have used this model since in general, diabetic mice have much greater hyperlipidemia than do controls (22). By using these animals, we hoped to gain insights into pathways for lipoprotein catabolism that are exclusive of the LDL receptor and that might lead to altered lipoprotein metabolism in humans. ApoB-containing lipoprotein clearance from the circulation occurs by at least three pathways: LDL receptor uptake, uptake via other receptors such as LRP (32) and clearance via associated with and perhaps uptake by cell surface proteoglycans (33). This latter pathway leads to lipoprotein trapping, a rapid plasma clearance within minutes rather than hours of lipoprotein particles via their electrostatic binding within the space of Disse in the liver. Recently, liver specific deletion of *Ndst1*, which is responsible for proteoglycan sulfation, led to hyperlipidemia, a process that was augmented when LDL receptors were deficient (34). This experiment confirmed the importance of the proteoglycan clearance pathway in lipoprotein metabolism.

Others have been able to illuminate the role of the LRP-mediated pathway in remnant lipoprotein clearance using *Ldlr*^{-/-} mice (35). We assessed the effects of diet, rates of apoB synthesis, expression of lipoprotein receptors and acute LDL removal in control and

diabetic mice. Our data show the following: 1) Loss of LDL receptors is associated with elevated fasting cholesterol levels after STZ-treatment. 2) This is associated with an increase in the size and lipid content of VLDL and LDL as well as the proportion of apoB48 in LDL. 3) Diabetic *Ldlr*^{-/-} mice do not have an increase in liver production of apoB during fasting or postprandially. 4) Diabetic mice have a marked increase in food intake, however, the increase in cholesterol consumed is not the major cause of hypercholesterolemia in these mice. 5) LDL from diabetic mice have reduced plasma clearance and this is associated with decreased liver “trapping” of the particles, increased circulating levels of apoE and decreased expression of *Ndst1*.

We sought to determine why, in many studies, diabetes elevated cholesterol in *Ldlr*^{-/-} mice. Because elevated cholesterol occurred on the *Ldlr*^{-/-} background, this suggested that reduced uptake occurred with lack of the normal LDL receptor pathway. Mice overexpressing human apoB do not show a similar hyperlipidemia with STZ-treatment (12). First, we characterized the lipoproteins and showed that apoB lipoproteins were larger in the diabetic mice. Second, we confirmed that despite greater food intake, STZ-treated *Ldlr*^{-/-} mice did not have increased liver production of lipoproteins. This was done by assessing radioactive methionine incorporation into apoB in mice in which lipolysis and removal of secreted lipoproteins were blocked by injection of detergent. Other receptors have been implicated in hepatic lipoprotein uptake in *Ldlr*^{-/-} mice (36) (37). Neither receptor (LRP or SR-BI) was reduced.

Unlike humans that only produce apoB48 in the intestine, both the liver and intestine produce apoB48 in the rodent. We did not find an increase in apoB48 production in the fasting mice, nor in the postprandial period when intestinal production would be most

robust. As a second means to assess intestinal lipoprotein production, we performed studies of intestinal absorption of retinyl ester in a triglyceride emulsion. Neither the amount of label nor the peak triglyceride found in the plasma differed between the control and diabetic mice.

STZ-treated mice consumed a greater amount of cholesterol, as their food intake increased ~60%. For this reason, we tested whether altering cholesterol intake and reducing the dietary drive to produce intestinal lipoproteins would correct the hyperlipidemia. Using half of the concentration of cholesterol in the chow to correct for the greater intake in the diabetic mice did not reduce plasma cholesterol levels to levels found in the non-diabetic mice.

The reasons for the increase in apoB-containing lipoproteins became apparent when we performed kinetic studies using labeled lipoproteins from control and STZ-treated mice. Lipoproteins from the diabetic mice were rapidly removed from the plasma of control mice and found within the liver of control mice. Such rapid loss of lipoproteins, in 10 minutes, is most compatible with “trapping” and not receptor-mediated uptake. This process is thought to be a non-receptor mediated association in which the basic residues of apoE on the surface of lipoproteins has an ionic interaction with sulfated proteoglycans. Livers from diabetic rodents have a defect in proteoglycans (38),(39), perhaps due to reduced expression of *Ndst1* (21). We confirmed reduced *Ndst1* expression in the STZ-treated *Ldlr*^{-/-} mouse livers and showed that hepatocytes cultured under hyperglycemic conditions also have reduced *Ndst1* expression. Recently, studies of a hepatocyte specific knockout of *Ndst1* confirmed the role of proteoglycan trapping in normal catabolism of apoB-containing lipoproteins (34).

Our current studies in *Ldlr*^{-/-} mouse should be contrasted with those exploring the

reasons for diabetes-induced hyperlipidemia in wild-type mice and mice expressing only apoB48 or apoB100 (7). In these models with intact LDL receptors, the apoB48-, but not apoB100-expressing mice had increased fasting lipids with diabetes. Presumably any increase in apoB100 lipoproteins was handled by the LDL receptor. In contrast, there was an increase in apoB48 lipoproteins in the diabetic wild-type and apoB48-only expressing mice. In this study, there was also a decrease in liver proteoglycans; liver proteoglycans are thought to capture circulating lipoproteins within the hepatic sinusoids (33). In our study, “trapping” of the LDL from diabetic mice was increased when these particles were injected into control, but not diabetic, mice. Thus, it appears that the STZ-treated animals accumulated a subclass of lipoproteins that contained apoE and that were normally rapidly cleared from the bloodstream.

Humans with type 1 and type 2 diabetes do not usually show a marked elevation of plasma lipid levels (40; 41). Whether the increased postprandial lipemia found in type 2 patients is due to defective proteoglycan clearance of plasma lipoproteins (as found in this type 1 model), altered apoB production in the liver due to increased fatty acid return and decreased apoB degradation, or reduced lipoprotein lipase activity is not clear. However, our animal model might mimic the hyperlipidemia found in some type 1 patients with poorly controlled diabetes. In this situation, LDL receptors may be reduced due to lack of insulin actions (42). In addition, intestinal overproduction of lipoproteins, which occurs in some diabetic animal models (43), might lead to a greater flux of remnant lipoproteins that require an initial “trapping” in the liver. Thus, a reduction in lipoprotein receptors, the high cholesterol diet fed to our animals but representative of the diet standardly eaten by humans in western society, and reduction of liver trapping of

lipoproteins may all contribute to the postprandial lipemia found in human diabetes (44).

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TABLE 1. Fasting glucose, lipid and lipoprotein levels in control and STZ-treated mice

Gene	STZ	Diet	Body Weight (g)	Glucose (mg/dL)	TG (mg/dL)	TC (mg/dL)
<i>Ldlr</i> ^{-/-}	+	0.15% HC	23 + 3	524 + 108 **	208 + 82	1696 + 478 **
	-	0.15% HC	26 + 2	151 + 15	125 + 19	884 + 134
<i>Ldlr</i> ^{-/-}	+	Chol/CA	25 + 3	357 + 72 **	200 + 80	3189 + 1022 **
	-	Chol/CA	25 + 2	155 + 21	158 + 51	1950 + 646
<i>Ldlr</i> ^{+/-}	+	0.15% HC	21 + 3 **	291 + 124	42 + 22	451 + 146 *
	-	0.15% HC	27 + 2	140 + 27	62 + 12	247 + 49

	TG	VLDL TC	LDL TG	LDL TC	HDL TG	HDL TC
STZ HC	134 + 88	999 + 308**	7 + 4	646 + 115*	1 + 8	38 + 25 *
Control HC	68 + 17	250 + 111	18 + 11	387 + 141	4 + 3	79 + 25

STZ=streptozotocin-treated diabetic mice; TG=triglyceride; TC=total cholesterol; HC=0.15% cholesterol diet; Chol/CA=cholesterol cholic acid diet. p<0.05; *, p<0.01; **, n= 4-8 in each group.

TABLE 2. Metabolic parameters of mice eating 0.15 and 0.075% cholesterol diets

STZ	Diet	Food Eaten (g/d/m)	Body Weight (g)	Glucose (mg/dL)	TG (mg/dL)	TC (mg/dL)
+	0.15% HC	5.47 + 1.31**	23 + 3 **	489 + 110**	217 + 14	2007 + 844**
-	0.15% HC	3.04 + 0.17	29 + 2	133 + 25	122 + 39	767 + 270
+	0.075 % HC	5.16 + 0.72 **	23 + 3	534 + 77**	109 + 26*	1697 + 629**
-	0.075 % HC	3.02 + 0.21	27 + 2	126 + 24	110 + 32	700 + 202

HC= high cholesterol diet; p<0.05; *, p<0.01; **, n = 12-16 in each group.

FIGURE LEGENDS

Figure 1. Distribution of cholesterol and triglyceride (TG) in the plasma of control and diabetic *Ldlr*^{-/-} mice after four weeks on 0.15% cholesterol diet. Serum was taken after 6 hours of fasting. 250 μ l of pooled plasmas (n=5-7) were analyzed by FPLC as described in methods. Closed circles represent control animals and open circles represent STZ-treated animals. A. Distribution of cholesterol; an expanded view of the HDL region is included in the inset. B. Distribution of triglyceride.

Figure 2. ApoB and apoE in plasma VLDL and LDL. Lipoproteins were isolated by sequential ultracentrifugation. A. VLDL and LDL were isolated by ultracentrifugation using an equal volume of plasma. ApoE was analyzed by 4-20% and apoB48 4% SDS-PAGE, respectively. ApoE/B was determined by densitometric scanning. B. Thirty μ g of protein were loaded into a 4% gel, transferred to nitrocellulose and blotted using antibodies to apoB, a representative blot (A), average ratios of LDL (n = 3-4)(B), VLDL (C). *P< 0.01. Solid bars represent control animals and clear bars represent STZ-treated animals.

Figure 3. ApoB production in STZ-treated mice. Fasting production of apoB was measured. Fasted mice were injected with [³⁵S]Promix and Triton WR1339. Blood was collected 60- and 120 min post-injection and plasma from each point was subjected to 4% SDS-PAGE and fluorography. A shows the autoradiogram from the 120-min time point and B the normalized and quantified data obtained by scintillation counting. C shows the increases in plasma TG in the two groups of mice. D. Postprandial apoB production was assessed as above in diabetic and non-diabetic *Ldlr*^{-/-} mice fed a cholesterol-containing diet for 4 weeks. After an overnight fast P-407 treated mice received a gavage of 100 μ l of [³⁵S]methionine containing corn oil. Labeled apoB in plasma was assessed by 4% SDS-PAGE.

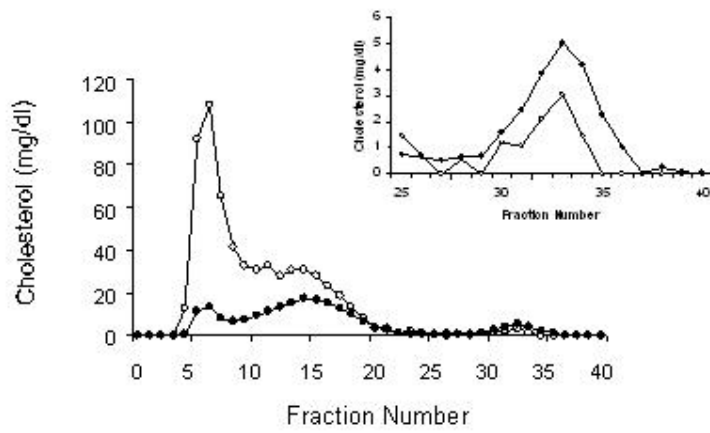
Figure 4. Expression of lipoprotein receptors in control and STZ-treated mice. Livers from control and diabetic mice were used for mRNA analysis and protein expression by Western blot analysis. A. Real time PCR measurements of LRP and SR-BI. B. Western blots.

Figure 5. Plasma clearance (A) and liver uptake (B) of LDL and VLDL. VLDL and LDL were isolated from STZ-treated *Ldlr*^{-/-} mice and labeled with ¹²⁵I. Labels were injected into control (open bars) and diabetic mice (solid bars) and plasma clearance and liver uptake after 10 minutes, an indication of trapping, were determined. * p<0.05.

Figure 6. Ndst1 gene expression. A. Effects of diabetes on hepatic Ndst1 mRNA levels in *Ldlr*^{-/-} mice. RNA was extracted from samples of liver and measured Ndst1 mRNA by quantitative real-time PCR. (*p<0.05, n=6-8/group). B. Effects of high glucose on Ndst1 mRNA levels in hepatoma cells in vitro. McArdle hepatoma cells were cultured for 24h with 5mM or 25mM glucose medium. (*p<0.05, n=6/group). Ndst1 mRNA normalized to β -actin mRNA.

Figure 1

A



B

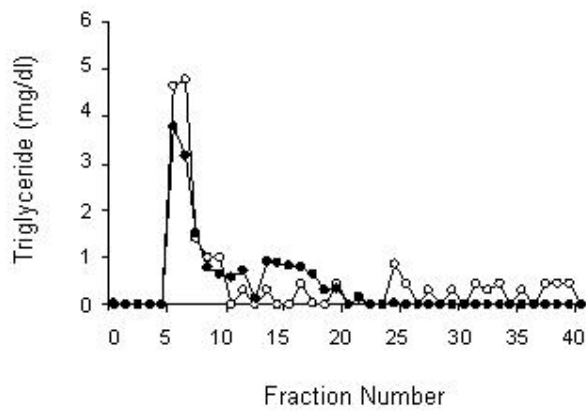
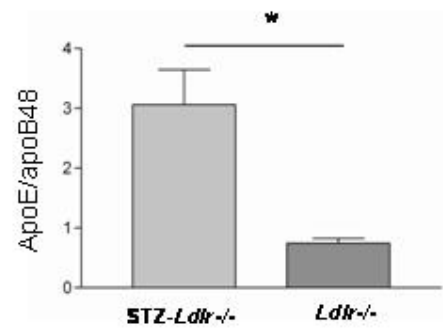
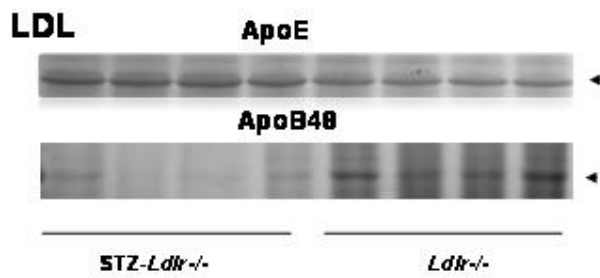
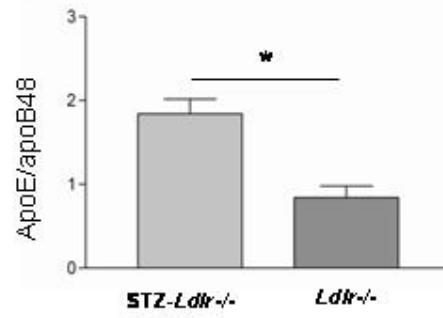
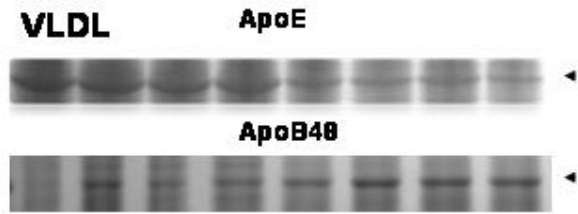


Figure 2

A



B

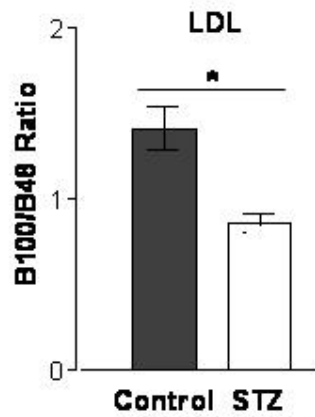
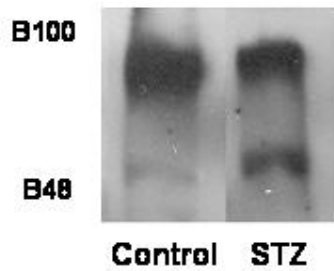


Figure 3

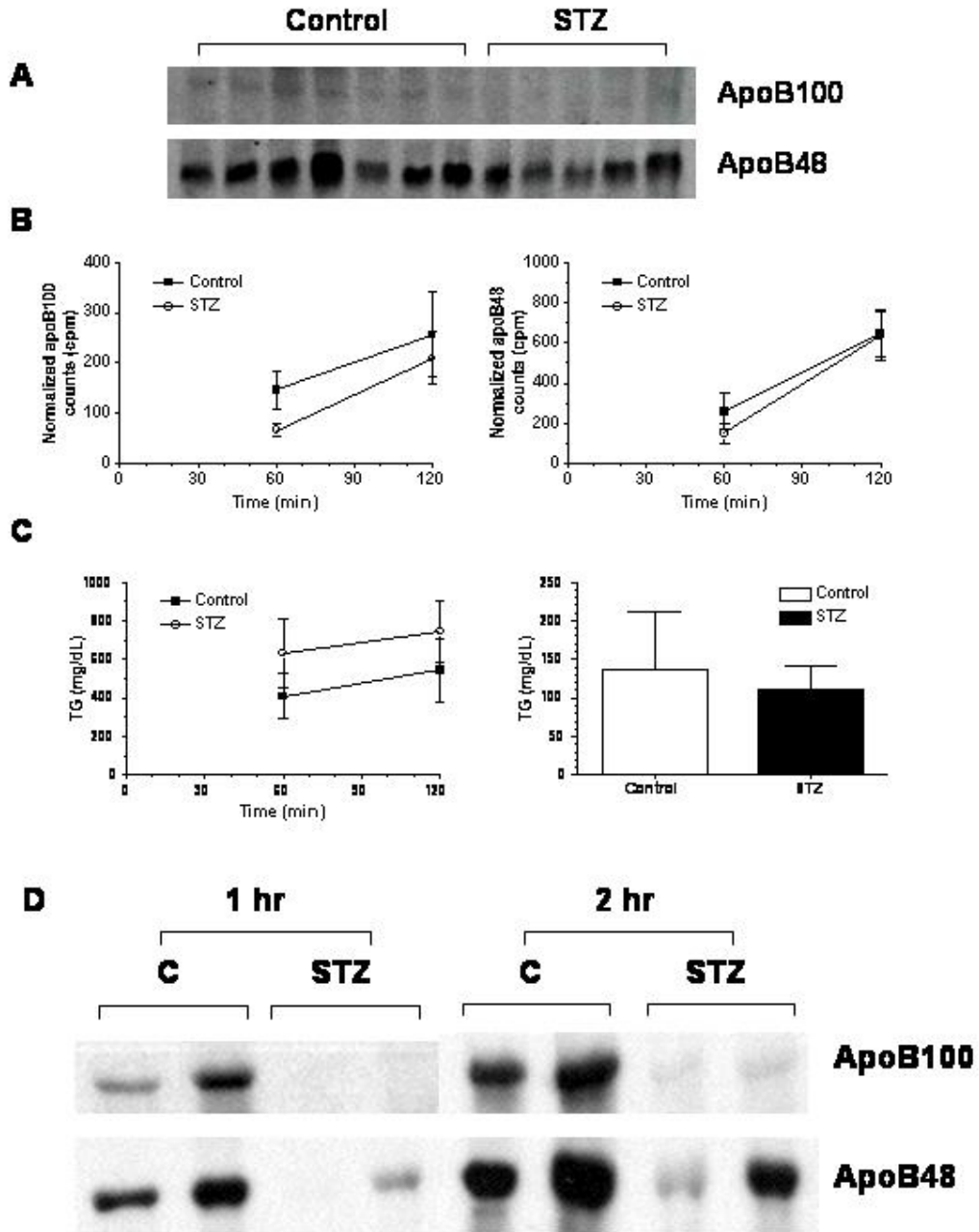


Figure 4

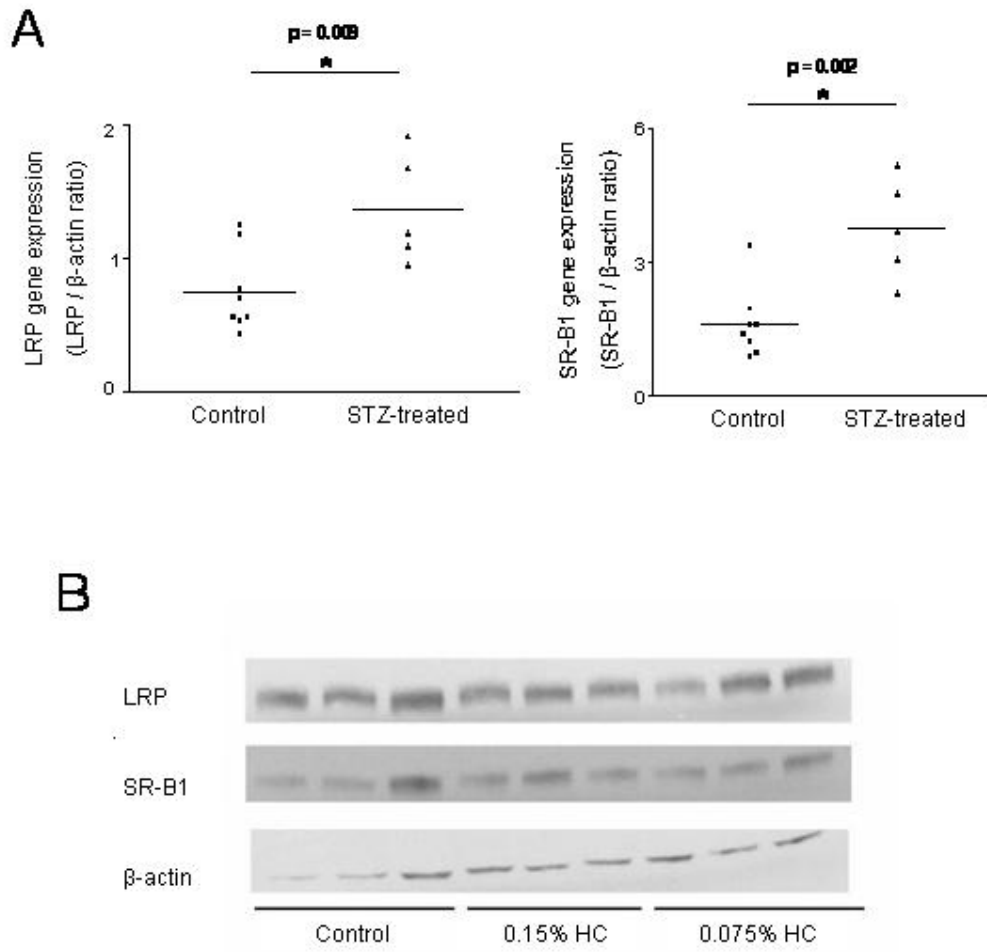


Figure 5

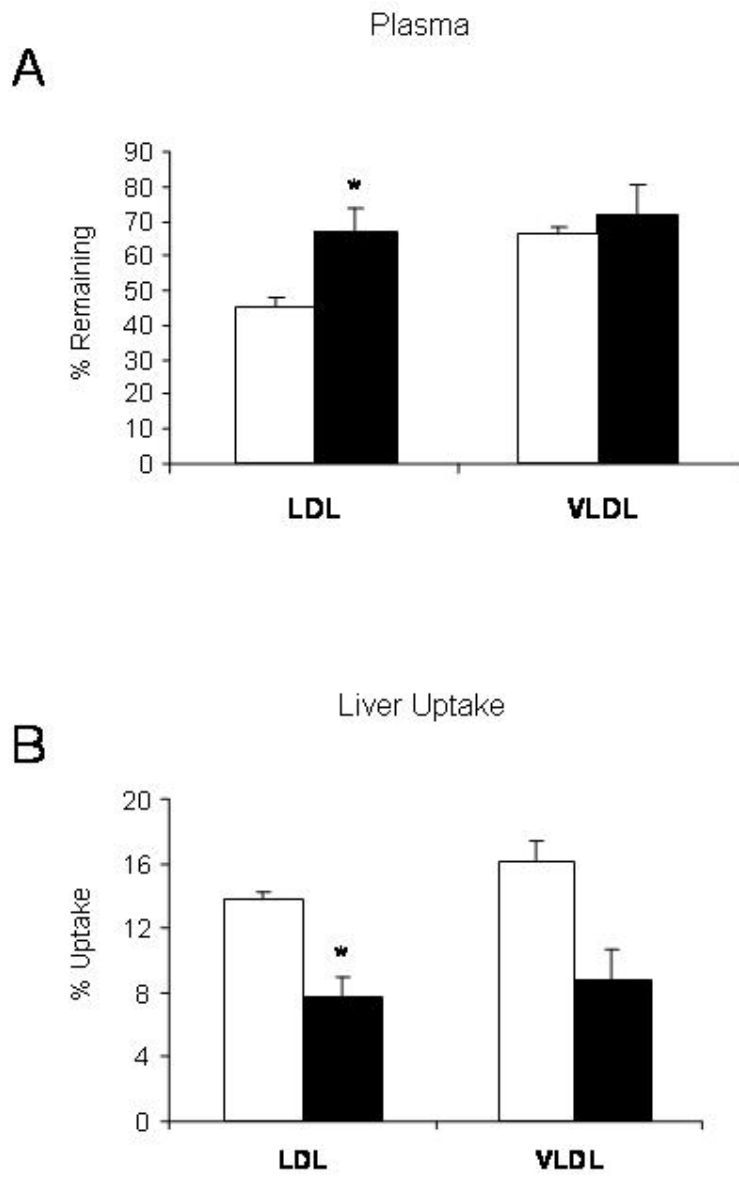


Figure 6

