Streptozotocin-induced diabetes in human apolipoprotein B transgenic mice: effects on lipoproteins and atherosclerosis

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Abstract The effects of diabetes and lipoprotein lipase (LpL) on plasma lipids were studied in mice expressing human apolipoprotein B (HuBTg). Our overall objective was to produce a diabetic mouse model in which the sole effects of blood glucose elevation on atherosclerosis could be assessed. Mice were made diabetic by intraperitoneal injection of streptozotocin, which led to a 2- to 2.5-fold increase in plasma glucose. Lipids were assessed in mice on chow and on an atherogenic Western type diet (WTD), consisting of 21% (wt/wt) fat and 0.15% (wt/wt) cholesterol. Plasma triglyceride and cholesterol were the same in diabetic and nondiabetic mice on the chow diet. On the WTD, male diabetic HuBTg mice had a >50% increase in plasma cholesterol and more very low density lipoprotein (VLDL) cholesterol and triglyceride as assessed by FPLC analysis. A Triton study showed no increase in triglyceride or apolipoprotein B production, suggesting that the accumulation of VLDL was due to a decrease in lipoprotein clearance. Surprisingly, the VLDL increase in these mice was not due to a decrease in LpL activity in postheparin plasma. To test whether LpL overexpression would alter these diabetes-induced lipoprotein changes, HuBTg mice were crossed with mice expressing human LpL in muscle. LpL overexpression reduced plasma triglyceride, but not cholesterol, in male mice on WTD. Aortic root atherosclerosis assessed in 32-week-old mice on the WTD was not greater in diabetic mice. In summary, diabetes primarily increased plasma VLDL in HuBTg mice. LpL activity was not decreased in these animals. However, additional LpL expression eliminated the diabetic lipoprotein changes. These mice did not have more atherosclerosis with diabetes.—Kako, Y., L-S. Huang, J. Yang, T. Katopodis, R. Ramakrishnan, and I. J. Goldberg. **Streptozotocin-induced diabetes in human apolipoprotein B transgenic mice: effects on lipoproteins and atherosclerosis. *J. Lipid Res*. 1999.** 40: **2185–2194.**

Supplementary key words triglyceride • cholesterol • apolipoprotein • heart • coronary

Diabetes mellitus is a major risk factor for the development of atherosclerosis and its clinical manifestations including coronary artery disease, stroke, and peripheral vascular disease. Several theories have been expounded for the atherogenic effects of diabetes. In vitro data suggest that hyperinsulinemia increases the growth of cells including macrophages (1) and smooth muscle cells (2) that are components of the atherosclerotic plaque. Hyperglycemia may have several deleterious actions. Advanced glycosylation end products have been postulated to increase inflammation, promote oxidation, cause autoimmune processes, and increase endothelial barrier permeability (3, 4).

A well-known consequence of diabetes is the development of lipoprotein abnormalities. Some diabetic patients have increased low density lipoprotein (LDL) cholesterol concentrations, which can be reduced with improved glycemic control (5). However, the most common abnormalities in humans with poorly controlled type 1 and 2 diabetes are hypertriglyceridemia and low high density lipoprotein (HDL) (6). Human metabolic studies show that overproduction of triglyceride is often found in patients with diabetes mellitus. This may be secondary to increased liver production of triglycerides from free fatty acids released from poorly insulinized adipocytes. A second etiology of diabetic hypertriglyceridemia is a reduction in the activity of lipoprotein lipase (LpL). This results in decreased triglyceride lipolysis in peripheral tissues such as adipose and muscle, and return of more triglyceride to the liver. LpL is stimulated by acute (7) and by chronic insulin therapy (8). Thus, diabetic hypertriglyceridemia is caused by at least two separate metabolic aberrations and it is likely that the most severe forms of this disorder involve both processes.

Atherosclerosis, the cause of most diabetic macrovascular diseases, has recently been studied in several geneti-

Abbreviations: apo, apolipoprotein; WTD, western type diet; STZ, streptozotocin; LpL, lipoprotein lipase; HuBTg, human apoB transgenic mouse; WT, wild-type; CTR, control.

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cally modified mice. Disruptions of the apolipoprotein (apo) E or the LDL receptor genes cause severe hypercholesterolemia and mimic human disorders with specific genetic hyperlipidemias and atherosclerosis (9). Human apoB transgenic mice (HuBTg) have increased cholesterol and triglyceride levels and develop atherosclerosis on high fat diets with (10, 11) or without added cholic acid (12). The effects of diabetes on lipoprotein profiles in this model are not known and were investigated. In addition, HuBTg mice were crossed with LpL overexpressing mice to assess whether the increase in LpL would correct any diabetes-induced hypertriglyceridemia. Our objective was to correct any diabetes-induced lipoprotein abnormalities and, thereby, determine whether hyperglycemia itself altered atherosclerosis. Finally, in a relatively small group of mice, we tested whether the diabetes would lead to a marked increase in atherosclerotic lesions.

METHODS

Transgenic mice

Human apoB transgenic mice and muscle-specific LpL-expressing transgenic mice (MCK-LpL) were generated as described (13, 14). HuBTg mice had a mixed genetic background of predominantly C57BL/6J (>75%) and FVB/N strains. Low expressing MCK-LpL mice that do not develop myopathy had a mixed genetic background of predominantly C57BL/6J (>75%) and CBA/J.

Mice were maintained in a temperature-controlled (25°C) facility with a 12-h light/dark cycle and given free access to food and water, except when fasting blood specimens were obtained. Mice were fed either laboratory rodent diet (PMI Nutrition International Inc., St. Louis, MO) or Western-type diet (WTD) (No. 88137, Teklad Premier Laboratory Diets, Madison, WI). Rodent chow contained 4.5% (wt/wt) fat and 200 ppm cholesterol; the WTD contained 21% (wt/wt) fat (polyunsaturated/saturated = 0.07), 0.15% (wt/wt) cholesterol, and 19.5% casein. These diets were free of sodium cholate. Blood samples were drawn from mice (anesthetized with methoxyflurane) by retroorbital phlebotomy into tubes containing anticoagulant (1 mm EDTA) using heparinized capillary tubes. Mice were allowed 7 days to recover between phlebotomies.

Genotyping

Transgenic mice were screened with primers 5-AGAAGGTTC CAGATGTCTATGAGG-3 and 5-TCCAAGTATCTGTCTTCAAC AAACC-3 for human apoB. PCR amplification was performed using 35 cycles at 94°C for 1 min and 55°C for 1 min and 72°C for 45 sec (13). Genotypes for MCK–human LpL mice were determined by PCR as described below. Human LpL specific primers used were as follows: 5-CCTCAAGGGAAAGCTGCCCAC-3 and 5-GTTACCGTCCAGCCATGGATCACCA-3. The protocol involved 30 cycles of 93°C for 1 min, 60°C for 1 min, and 72°C for 2 min (15). PCR products were resolved by gel electrophoresis, and were visualized by ethidium bromide staining.

Diabetes

Mice were made diabetic by streptozotocin (STZ) treatment similar to that described by Kunjathoor, Wilson, and LeBoeuf (16). The schedule of treatment is shown in **Fig. 1**. Mice were divided into two groups; half were treated with STZ (Sigma Chemical Co., St. Louis, MO). STZ was dissolved in sterile citrate buffer (0.05 m, pH 4.5, 3 mg/ml) and used within 20 min of preparation. The solution was injected intraperitoneally into mice (40 mg/kg, ~400 μl) for 5 consecutive days during the second week of the study. To avoid hypoglycemia, mice were continued on a chow diet for 4 weeks after the first treatment but then switched for 2 weeks to the WTD. Six weeks after the first injection, the mice were switched back to chow for 2 weeks and a second STZ treatment was given to maintain hyperglycemia. This protocol, illustrated in Fig. 1, was used to maximize the amount of time in which the mice consumed the WTD.

Plasma glucose, lipid, and lipoprotein determinations

Glucose was measured using a kit from Sigma Diagnostic (Glucose trinder kit #315), and lipid assays were measured using kits from Boehringer Mannheim Biochemicals (Indianapolis, IN). 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 from 22- to 32-week-old mice in a TLA 100 rotor (Beckmann Instruments, Palo Alto, CA).

Gel filtration chromatography

Pooled fasting plasma (22–32 weeks old, 3–7 mice per each group) (500 μl) was applied to a combined column system composed of two Superose 6 columns in series (FPLC; Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Eighty 0.5-ml fractions were collected. Cholesterol and triglyceride levels of FPLC fractions were measured using enzymatic reagents in colorimetric assays modified for a 96-well plate spectrophotometer (SpectraMax 250; Molecular Devices, Sunnyvale, CA) as described previously (17).

ApoB production rates

Five 32-week-old mice from each group were used to determine triglyceride and apoB production rates using previously reported methods (12). Briefly, mice were bled to measure baseline triglyceride levels and were then injected with a solution containing 15% Triton WR-1339 (0.5 mg/g body weight; Sigma) in 0.9% NaCl and 100 μCi of [^{35}S]methionine (Amersham Life

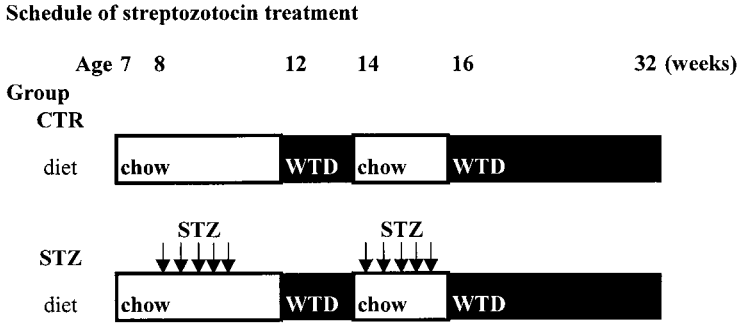

Fig. 1. Diet and STZ treatment protocol. Mice were entered into the study at 7 weeks of age. These mice were divided into two groups: streptozotocin (STZ) and control (CTR). The STZ group was injected with 40 mg/kg of STZ intraperitoneally for 5 days at 8 and 14 weeks of age. To avoid hypoglycemia, the STZ mice were fed chow diet until 12 and 14–16 weeks of age. WTD was used at 12–14 and 16–32 weeks of age. The CTR group did not receive treatment of STZ, and was fed the same diet as the STZ group.

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Science Inc., Arlington Heights, IL). Blood was collected at 60 and 120 min after injection. Plasma triglyceride levels were then assayed. ApoB proteins were separated in 4% SDS-PAGE followed by autoradiography. Both apoB-100 and apoB-48 protein bands were cut out from dried gels and counted in scintillation fluid.

Postheparin lipases

Postheparin plasma was obtained from 4–6 mice in each group that were 21- to 32-weeks old. The animals were injected with 10 units of heparin (Elkins-Sinn, Inc., Cherry Hill, NJ) intravenously via a tail vein. Blood was obtained by retroorbital phlebotomy 5 min later and plasma was frozen at −70°C within 30 min. Postheparin lipase activities were measured using the glycerol-based emulsion described by Nilsson-Ehle and Schotz (18) with heat-inactivated human serum as a source of apoC-II. Measurements of human LpL activity were obtained by inhibition with a monoclonal antibody (19), and total LpL was assessed using a chicken antiserum that inhibits both human and mouse LpL (20). The data from different assays were normalized with a standard human postheparin plasma activity.

Quantitative atherosclerosis analysis

Mice were killed at 32 weeks of age and atherosclerosis assays were performed on the aortic roots as described previously (12). In brief, hearts were perfused with PBS, fixed in 10% phosphate-buffered formalin, embedded in OCT compound, and sectioned at 10 μm thickness in a cryostat. Sections were stained with Oil Red O and hematoxylin, and counter-stained with light green. Lesions, of the proximal aorta, stained with Oil Red O were measured in 80-μm intervals and the mean lesion area per section was calculated. As lesion area data are typically skewed with a few large values that make the mean much higher than the median, a square-root transformation was performed, scaled (by $2/\sqrt{\pi}$) to represent the lesion diameter in microns. Results are given for lesion areas and diameters. Statistical analyses were done by a multi-way analysis of variance with gender, LpL, and diabetes as the three factors.

Statistics

Analyses were performed using Student's *t* tests, except for the atherosclerosis data, and two-tailed *P* values are reported.

RESULTS

Streptozotocin-induced diabetes in HuBTg mice

The effects of STZ injections, given at age 8 and 14 weeks, on glucose levels in male and female HuBTg mice are shown in **Fig. 2**. Figure 2A shows that severe diabetes was induced in STZ-treated male mice; glucose levels were an average of 18.1 ± 6.3 mmol/l at 12 weeks of age while the mice were consuming chow. At 20 weeks of age, while on a WTD, glucose was 30.8 ± 3.1 mmol/l. Thereafter, the elevated glucose levels remained constant from 20 to 32 weeks.

STZ-induced diabetes was less severe in female mice. In STZ-treated female mice, the mean glucose level on chow was 12.2 ± 2.0 mmol/l at 12 weeks. After switching to the WTD glucose was 23.5 ± 5.8 mmol/l at 20 weeks of age.

To determine whether the diets affected glucose after the second STZ injection, glucose levels at 16 weeks while on chow and 20 weeks while on WTD were compared. In STZ-treated male mice, glucose levels were identical at these two time points. In STZ-treated female mice, however, plasma concentrations of glucose were significantly higher ($P = 0.007$) in the 20-week-old WTD-fed mice (23.5 ± 5.8 mmol/l) than in the 16-week-old chow-fed mice (18.1 ± 3.0 mmol/l). Females, as shown in Fig. 2, were more resistant to the first STZ injection. Therefore, the increase in glucose between age 16 and 20 weeks could have resulted from the diets or from a more delayed response of the STZ injection in females.

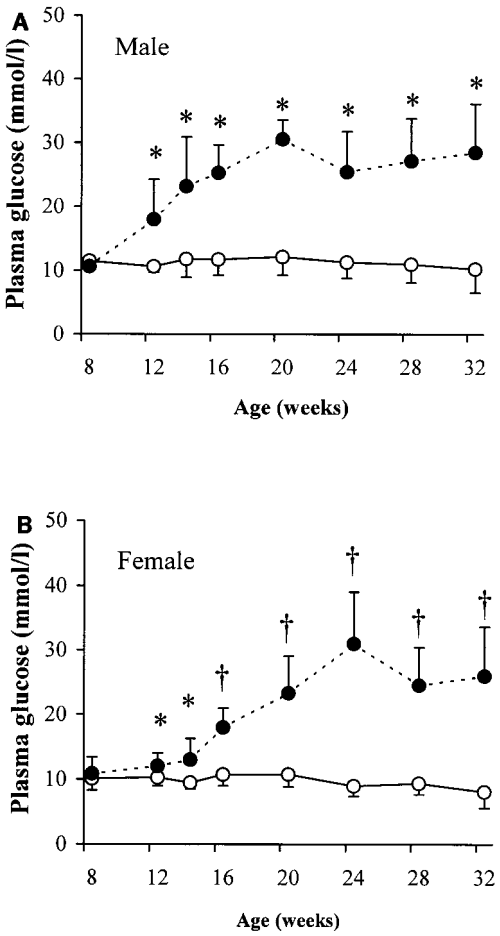

Fig. 2. Plasma glucose concentration of HuBTg mice. Control (open circle) and diabetic (closed circle) mice were fed either normal chow diet or WTD. Plasma glucose was determined colorimetrically as described in Methods. Values are reported as mean ± SD, n = 8–19 mice. Differences between control and diabetic mice were analyzed by Student's *t* test. * $P < 0.01$, † $P < 0.001$.

Effects of severe diabetes on body weight

Severe insulin-deficient diabetes is expected to reduce body weight gain. As shown in **Fig. 3A**, this effect was evident in the male mice. Beginning at 14 weeks of age, the male diabetic mice had less body weight gain than control mice. In females, control and diabetic mice had similar body weights up to 20 weeks of age (Fig. 3B). However, by 28 weeks of age, the diabetic females weighed significantly less than their control littermates, 24.5 ± 1.8 versus 28.1 ± 4.5 g, $P = 0.006$.

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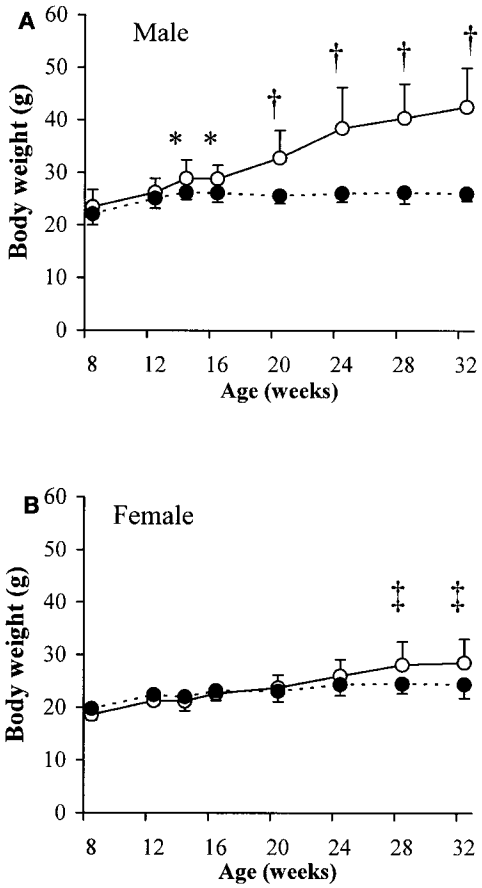

Fig. 3. Body weights of control and diabetic HuBTg mice. Nine to 19 mice from each group were weighed every 4 weeks. Weights of control mice are shown in the open circles and weights of diabetic mice in the closed circles. Differences between control and diabetic mice were analyzed by Student's *t* test. * $P < 0.05$, † $P < 0.001$, ‡ $P < 0.01$.

TABLE 1. Lipids in control and diabetic HuBTg mice

	Male		Female	
	Control n = 9	Diabetes n = 9	Control n = 10	Diabetes n = 13
	mmol/l		*mmol/l*	
Cholesterol				
Chow	2.7 ± 0.4	3.0 ± 0.4	3.7 ± 0.5	3.4 ± 0.5
WTD	8.8 ± 1.3[a]	14.0 ± 4.6[b,c]	9.1 ± 1.4[a]	8.7 ± 1.3[a]
Triglyceride				
Chow	1.9 ± 0.4	1.9 ± 0.5	1.7 ± 0.4	2.1 ± 0.6
WTD	2.3 ± 0.7	2.5 ± 1.2	1.0 ± 0.5[d]	1.6 ± 0.6

Data were obtained from 6-week-old mice fed chow and 20-week-old mice fed the WTD. Differences between control and diabetic mice were analyzed by Student's *t*-test. Values are reported as means ± SD.

[a] $P < 0.0001$ versus chow.
[b] $P < 0.001$ versus chow.
[c] $P < 0.05$ versus control.
[d] $P < 0.01$ versus chow.

Effects of chow and Western diets on plasma lipid levels

Diabetes in HuBTg mice on chow diets led to no appreciable changes in lipoprotein profiles. As shown in **Table 1**, 16-week-old male and female diabetic mice had cholesterol levels similar to those of control mice that were fed a chow diet. Male control and diabetic mice also had identical triglyceride levels (1.9 ± 0.4 mmol/l versus 1.9 ± 0.5 mmol/l). Female diabetic mice had a small, but not statistically significant, increase in triglyceride levels compared to female control mice (2.1 ± 0.6 mmol/l versus 1.7 ± 0.4 mmol/l).

Institution of the WTD markedly altered plasma lipids; mice eating the WTD had more than double the plasma cholesterol level of similar mice on chow. In male control mice, cholesterol levels increased from 2.7 ± 0.4 mmol/l to 8.8 ± 1.3 mmol/l and in females, cholesterol levels increased from 3.7 ± 0.5 mmol/l to 9.1 ± 1.4 mmol/l. There was no statistically significant difference in triglyceride levels in males, while in females, triglyceride declined on the WTD. Diabetes exacerbated the hypercholesterolemia in WTD-fed mice. This effect was most evident in the males. The cholesterol levels in the diabetic male mice were >50% greater than those in the control males, average of 14.0 versus 8.8 mmol/l. Triglyceride levels were not significantly increased in either male or female diabetic mice relative to control mice on either diet. HDL cholesterol was measured in a subgroup of these mice consuming a WTD. Fasting HDL levels in diabetic mice (3.2 ± 0.7 mmol/l; n = 5) were not significantly different from that in the control mice (3.3 ± 0.3 mmol/l; n = 3). Therefore, the only diabetes-induced change in plasma lipids was an increase in cholesterol in male mice consuming a WTD.

Lipoprotein profiles by FPLC

To better assess any changes in lipoproteins due to diabetes, plasma was pooled from 3 to 7 male mice (age 22–32 weeks) and used for FPLC analysis. As shown in **Fig. 4A**, an increase in VLDL cholesterol was found in diabetic HuBTg mice when compared to the control HuBTg mice. In previous reports (11) it was noted that most of the plasma triglyceride in HuBTg mice was in LDL-sized particles. As shown in Fig. 4B, this was also true of our mice. The major triglyceride peak totally overlapped that of LDL cholesterol shown in Fig. 4A. Diabetic HuBTg mice also had a peak of triglyceride in the VLDL-sized particles. Therefore, STZ treatment caused a shift of triglyceride from LDL- to VLDL-sized particles.

Effect of the MCK-LpL transgene on lipids

To assess the effect of LpL overexpression on diabetes-induced changes in lipoprotein profiles, MCK-LpL transgenic mice were bred with HuBTg mice and treated with the same diabetes-inducing protocol used in the HuBTg mice. **Figure 5** shows that glucose levels were similar in STZ-treated male and female HuBTg mice with or without the MCK-LpL transgene. These data showed that there was no significant glucose altering effect by LpL overexpression in this mouse model.

The body weight gains were similar in STZ-treated HuBTg mice expressing the MCK-LpL transgene when compared to STZ-treated HuBTg mice (data not shown). Although LpL overexpression in muscle has been reported

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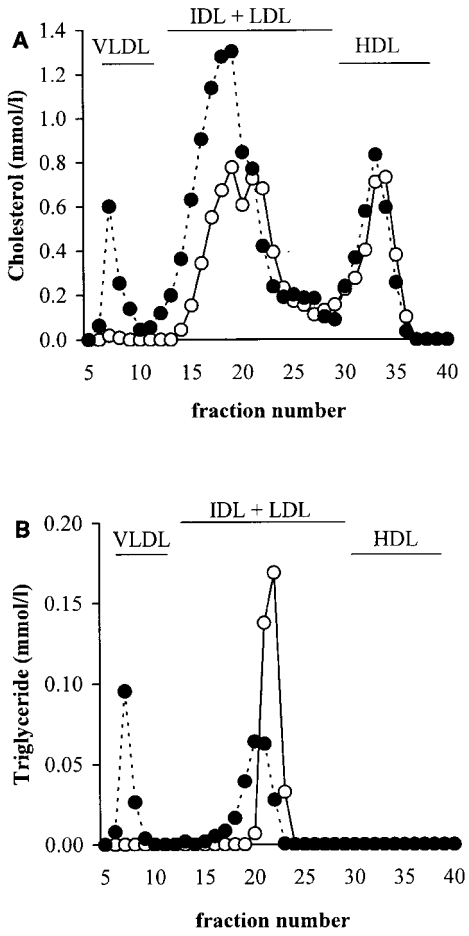

Fig. 4. Distribution of cholesterol (A) and triglyceride (B) in the plasma of male control (open circle) and diabetic HuBTg mice (closed circle). Five hundred μl of fasting pooled plasma from 3–6 mice consuming a WTD were analyzed by FPLC (gel filtration chromatography). Fractions (0.5 ml) were collected. VLDL elutes in fractions 5 to 10, IDL and LDL in fractions 10 to 29, and HDL in fractions 29 to 37. Values were normalized using the total cholesterol and triglyceride concentration of the pooled sample to correct for recovery.

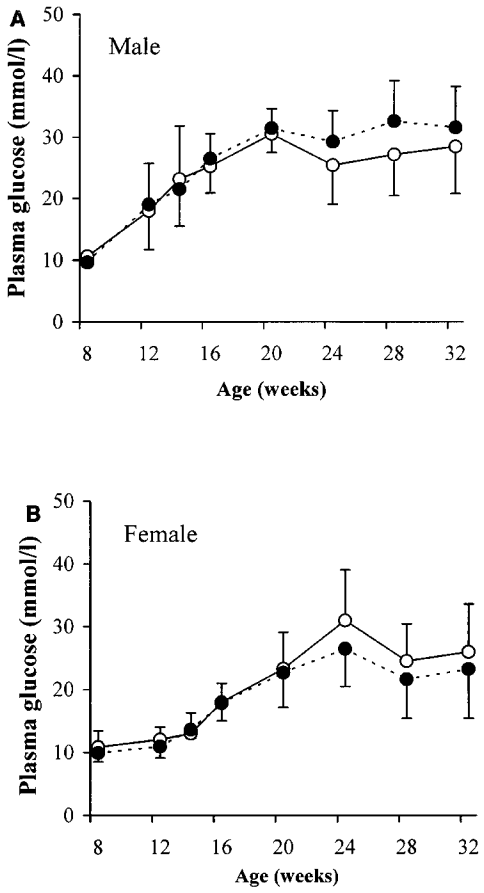

Fig. 5. Plasma glucose concentration of diabetic mice. Plasma glucose of diabetic HuBTg (open circle) and HuBTg/MCK-LpL mice (closed circle) was determined colorimetrically as described in Methods. Values are reported as mean ± SD, n = 5–24 mice. Differences between control and diabetic mice were analyzed by Student's *t* test.

to cause myopathy and weight loss (14), we used a low expressing MCK-LpL transgene that did not alter growth of the STZ-treated male and female mice.

As expected, the MCK-LpL transgene produced several alterations in lipoprotein profiles (**Table 2**). Triglyceride levels in chow-fed MCK-LpL mice were the same as in HuBTg mice without the MCK-LpL transgene. A 40% reduction in triglyceride was noted in male mice on the WTD without a change in cholesterol (Table 2). In female mice, no significant differences were observed in cholesterol levels between HuBTg and HuBTg/MCK-LpL mice on either diet. As reported previously (21), HDL cholesterol was significantly less in MCK-LpL transgenic mice than in HuBTg mice. In males on the WTD, HDL decreased from an average of 3.3 ± 0.3 mmol/l to 1.9 ± 0.3 mmol/l, a 42% reduction, $P = 0.00012$. In females the HDL decrease from 2.6 ± 0.8 mmol/l to 1.6 ± 0.3 mmol/l with the MCK-LpL transgene was not statistically significant ($P = 0.094$) with this small number of mice.

Triglyceride levels in chow-fed HuBTg/MCK-LpL diabetic mice were 37% and 30% less than non-diabetic HuBTg mice (1.2 ± 0.4 mmol/l versus 1.9 ± 0.4 mmol/l in males and 1.2 ± 0.5 mmol/l versus 1.7 ± 0.4 mmol/l in fe-

TABLE 2. Lipids in HuBtg/MCK-LpL mice

	Control		Diabetic	
	Male n = 14	Female n = 10	Male n = 6	Female n = 7
	mmol/l		*mmol/l*	
Cholesterol				
Chow	2.9 ± 0.6	3.7 ± 0.3	2.9 ± 0.6	3.5 ± 0.4
WTD	8.2 ± 1.6	7.9 ± 1.2	11.6 ± 3.8	8.5 ± 1.8
Triglyceride				
Chow	1.6 ± 0.3	1.6 ± 0.2	1.2 ± 0.4[a]	1.2 ± 0.5[b,c]
WTD	1.4 ± 0.5[a]	1.0 ± 0.5	1.7 ± 0.4	1.0 ± 0.3

Data were obtained from 6-week-old mice fed chow and 20-week-old mice fed the WTD. Differences between control and diabetic mice were analyzed by Student's *t*-test. Values are reported as means ± SD.

[a] $P < 0.01$ versus HuBTg.
[b] $P < 0.05$ versus HuBTg.
[c] $P < 0.05$ versus HuBTg, DM.

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males, Tables 1 and 2). When the diabetic HuBTg/MCK-LpL mice were compared to diabetic HuBTg mice, the mice with human LpL had lower triglyceride levels; this was only statistically significant in the female mice (the SD was greater in the diabetic male mice). There were no differences in cholesterol and triglyceride levels between diabetic HuBTg and HuBTg/MCK-LpL mice consuming WTD.

The lipoprotein analysis by FPLC showed that the triglyceride reduction in the diabetic mice was due to a change in VLDL. The expression of the MCK-LpL transgene in male diabetic HuBTg mice reduced the VLDL cholesterol (**Fig. 6A**) and almost totally eliminated the VLDL triglyceride (Fig. 6B). These profiles also confirmed the reduction in HDL cholesterol found with isolated lipoproteins. Therefore, excess LpL converted the diabetic lipoprotein profile in STZ-treated HuBTg back to that of untreated HuBTg mice.

ApoB and triglyceride production

The increase in VLDL concentrations in the diabetic mice might have occurred due to either of two mechanisms: VLDL overproduction or defective VLDL catabolism. ApoB and triglyceride production rates were determined after Triton injection into control and diabetic mice. Triton WR1339 prevents lipoprotein catabolism and allows one to determine liver production of lipoproteins. Triglyceride production in mmol/l/h was calculated by subtracting the pre-Triton plasma triglyceride from that at 120 min. As shown in **Fig. 7A**, male HuBTg mice produced the greatest amount of triglyceride. Diabetes reduced triglyceride production; HuBTg/MCK-LpL mice produced significantly less triglyceride than did the same mice without diabetes. In addition, diabetic HuBTg/MCK-LpL mice produced significantly less triglyceride than diabetic HuBTg mice, presumably because more plasma triglyceride was metabolized in peripheral tissues and not recycled through the liver. In females there were no differences in triglyceride production rate among the four groups of mice (Fig. 7A). Production of apoB-100 was virtually identical in all four groups of mice (Fig. 7B); apoB-48 production also did not differ among

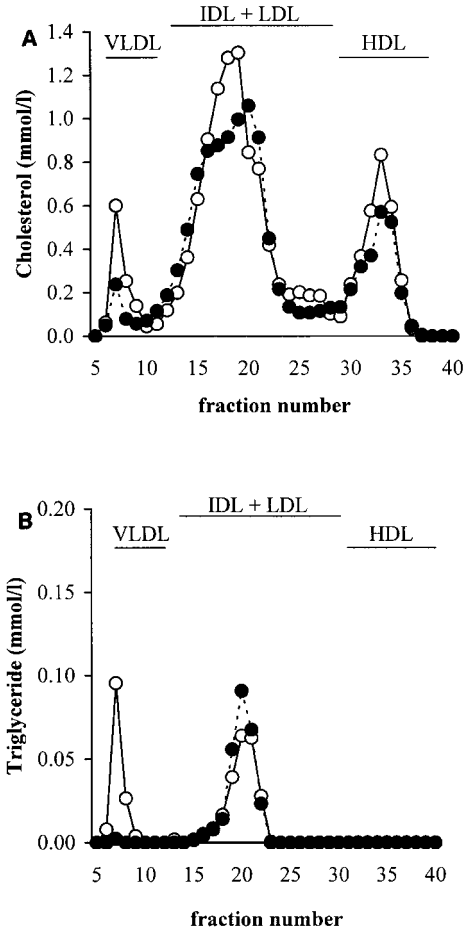

Fig. 6. Distribution of cholesterol (A) and triglyceride (B) in the plasma of male diabetic HuBTg (open circle) and HuBTg/MCK-LpL (closed circle) mice. Fasting pooled plasmas from 6–7 mice on WTD were analyzed by FPLC (gel filtration chromatography). Fraction volume was 0.5 ml. Elution volumes of the lipoproteins are as described in Fig. 4. Values were normalized to correct for recovery using the total cholesterol and triglyceride concentration of the pooled sample.

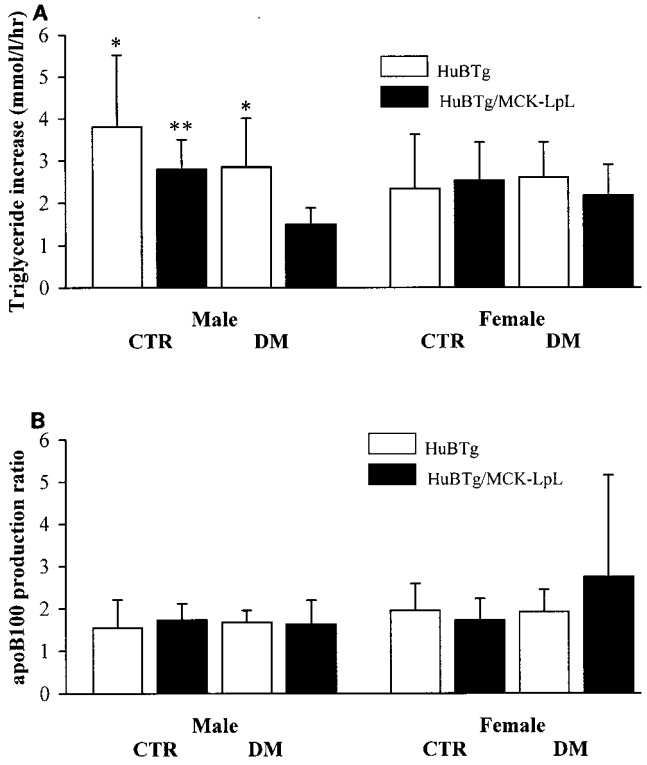

Fig. 7. In vivo triglyceride production (A) and apoB100 production (B). Five male and five female mice from each group were fasted for 6 h and injected with Triton WR1339 (0.5 mg/g body weight) and 100 μCi of [^{35}S]methionine. Mice were bled immediately prior to injection, and 60 min and 120 min after injection. Triglyceride levels were measured and the increase of triglyceride from baseline to 120 min was calculated. ApoB-100 production was estimated by dividing the amount of apoB-100 radioactivity at 120 min by that at 60 min. ApoB-100 radioactivity was assessed in the apoB-100 bands separated by SDS-PAGE. Open bars indicate HuBTg mice and closed bars indicate HuBTg/MCK-LpL mice. Values are reported as mean ± SD. Differences between the genotypes were assessed by Student's *t* test. * $P < 0.05$ vs. diabetic HuBTg/MCK-LpL mice; ** $P < 0.01$ vs. diabetic HuBTg/MCK-LpL mice.

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the mice (data not shown). These data suggest that the diabetic mice did not produce more triglyceride-rich apoB lipoproteins.

Postheparin LpL activity

Because of the limited effects of the MCK-LpL transgene on blood lipids compared to those in other reports (22), we speculated that there may have been a defect in production of this enzyme. As much of the regulation of LpL is at the posttranslational level (23), we were especially concerned that the diabetes in these mice might have prevented production of active LpL in the muscle. To assess this possibility, both human and mouse LpL activities were measured in postheparin plasma using antibodies that inhibited both mouse and human LpL or just human LpL (**Fig. 8**). Human LpL was found in the postheparin plasma of both male and female HuBTg/MCK-LpL mice at activity levels that were approximately equal to or greater than those of mouse LpL. Diabetes did not decrease LpL activities. Neither human nor mouse LpL was reduced in male and female mice that were made diabetic. Therefore, diabetes did not alter LpL production.

Atherosclerosis

In a limited number of mice, assays of atherosclerosis in the aortic root were performed and the data are shown in **Table 3**. The statistical analysis was performed using the lesion diameters. As expected, female mice had more atherosclerosis than males (158.2 ± 11.9 μm versus 94.8 ± 25.2 μm, with corresponding mean lesion areas of 20,110 and 9,052 μm^2); this gender difference has been found in many studies of atherosclerosis in mice. When the females were analyzed both for the effects of diabetes and LpL gene expression, two significant results were found. Surprisingly, the diabetic mice had less atherosclerosis; the diameter of lesions in diabetic mice (HuBTg and HuBTg/MCK-LpL) averaged 109 μm versus 145 μm in non-diabetic mice ($P = 0.019$). LpL expression also reduced atherosclerosis; the diameter of lesions in MCK-LpL mice (diabetic and non-diabetic) averaged 112 μm versus 142 μm in HuBTg mice ($P = 0.045$). No significant effects of diabetes or LpL expression were found in the male mice.

TABLE 3. Atherosclerotic lesions in HuBTg mice with diabetes

Group	Gender	n	Mean Lesion Area	Diameter
			μm^2	μm
HuBTg	male	5	9052 ± 4851	94.8 ± 25.2
HuBTg/MCK-LpL	male	5	5464 ± 4142	65.1 ± 26.1
HuBTg, DM	male	5	12809 ± 8579	105.9 ± 35.7
HuBTg/MCK-LpL, DM	male	4	8994 ± 4726	95.6 ± 27.8
HuBTg	female	5	20110 ± 2909	158.2 ± 11.9
HuBTg/MCK-LpL	female	5	14215 ± 2814	131.6 ± 14.0
HuBTg, DM	female	5	13565 ± 3767	125.6 ± 19.4
HuBTg/MCK-LpL, DM	female	5	6988 ± 1441	92.3 ± 9.8

A total of 6 sections for each mouse (80 μm interval) were analyzed. The area of intimal Oil Red O-positive staining was measured in each section and the mean lesion area per section was calculated. The areas and diameters are reported as mean μm^2 ± SEM and mean μm ± SEM. When square root transformations of the atherosclerosis data were combined and used for a multi-way analysis of variance, diabetic female, but not male, mice had less atherosclerosis ($P = 0.019$). LpL expression also reduced atherosclerosis in female mice ($P = 0.045$).

DISCUSSION

Circulating lipoprotein profiles in wild type mice are markedly different from those in humans. ApoB-containing lipoproteins, VLDL and LDL, are the major cholesterol and triglyceride carriers in fasting human plasma. Mouse plasma has almost an order of magnitude fewer apoB lipoproteins than human plasma, and the major cholesterol-transporting lipoprotein in the mouse is HDL. This difference in lipoproteins underlies the normal resistance of mice to atherosclerosis, an apoB-initiated pathological process.

The HuBTg mice used for our investigation express human apoB in the liver and have levels of plasma apoB that are similar to those in human plasma (11). We showed that STZ-induced diabetes did not lead to a major change in lipoproteins in chow-fed HuBTg mice. Only male mice on a Western, non-cholic acid-containing diet had a significant increase in cholesterol after the induction of diabetes. The primary lipoprotein abnormality in human diabetes is an increase in VLDL (24) rather than in LDL; this lipoprotein alteration was reproduced in the STZ-treated mice. Gel filtration profiles of the diabetic

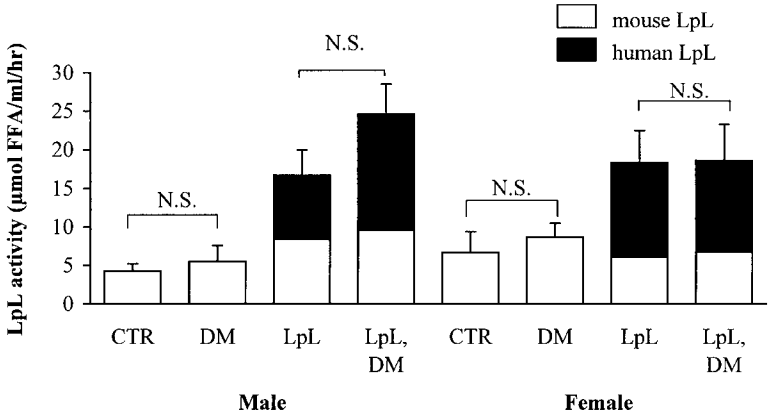

Fig. 8. Postheparin plasma LpL activity of control and diabetic HuBTg and HuBTg/MCK-LpL mice. Postheparin plasma was collected and LpL activity was measured as described in Methods. Mouse LpL activity was determined by subtracting human LpL activity from total LpL activity. Open bars indicate mouse LpL activity and closed bars indicate human LpL activity. Values are reported as mean ± SE. Differences between the groups were assessed by Student's *t* test.

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HuBTg mice showed the presence of a distinct VLDL peak that was lacking in non-diabetic mice. We then sought to determine the mechanism for the VLDL increase by assessing triglyceride and apoB production rates. Neither apoB nor triglyceride production increased significantly, as assessed by the radiolabeling of newly synthesized apoB and by the changes in plasma triglyceride levels after a Triton injection. Because production was not increased, VLDL catabolism must have been reduced.

VLDL is normally converted to LDL via the actions of postheparin lipases. LpL is the rate-limiting step in this process. In addition, some VLDL undergoes partial lipolysis and conversion to remnants that are then removed by the liver. To assess whether STZ-induced diabetes reduced LpL, LpL activity was measured in postheparin blood. We found that diabetes did not significantly alter LpL activity. This was surprising because LpL activity is reduced in diabetic humans (24). However, previous data from Bar-on, Chen, and Reaven (25) showed that STZ-induced diabetes in rats also failed to decrease postheparin lipase activity. Kinetic analyses suggested that the hypertriglyceridemia in their rats was not due to VLDL overproduction, and these investigators postulated that a lipase inhibitor was present in the plasma. Our data were similar and, as in rats, diabetic mice might have increased levels of a lipase-inhibiting substance in the blood. Several candidates, apoC-III (26), apoC-I (27), and apoE (28), alter plasma clearance of triglyceride-containing lipoproteins. The apoC-III gene is thought to have an insulin responsive element (29), and more apoC-III may be produced with insulin deficiency.

The expression of the MCK-LpL transgene virtually eliminated the VLDL peak observed in diabetic HuBTg mice. We presume that the added LpL overwhelmed any defect in VLDL catabolism and increased lipolysis and VLDL remnant removal. LpL overexpression reduced plasma lipoproteins in other diabetic mice. Shimada et al. (22) reported that diabetes-mediated increases in triglyceride were eliminated by overexpression of LpL. Cholesterol levels were also decreased by LpL overexpression. LpL in these mice was expressed using a CMV enhancer/chicken β-actin promoter. This leads to LpL overexpression in multiple tissues including the liver. Liver LpL increases hepatic lipoprotein uptake (20); this may occur via LpL serving as a ligand for the LRP family of receptors or via cellular internalization of LpL–lipoprotein complexes associated with cell surface proteoglycans (30). In the current studies, the MCK-LpL transgene did not reduce cholesterol in the HuBTg mice. Therefore, LpL expression in the liver or a defect in the normal repertoire of hepatic receptors may be required to demonstrate LpL-mediated effects on cholesterol-rich lipoproteins. In support of this hypothesis, Semenkovich, Coleman, and Daugherty (31) reported that fat-fed LDL receptor knockout mice that were also heterozygous for a knockout of the LpL gene had higher concentrations of both triglyceride and cholesterol.

LpL activity is low in patients with diabetes and is increased with insulin therapy (32). We had expected to find a similar reduction in postheparin plasma LpL in diabetic mice. Neither human LpL, produced via the non-insulin regulated MCK-LpL transgene, nor endogenous mouse LpL was reduced in diabetic mice. These data suggest that LpL regulation is different in humans and mice. The lack of diabetes-induced LpL reduction could underlie the relative resistance of mice to diabetes-induced hypertriglyceridemia. LpL regulation may also relate to variations in hypertriglyceridemia found in humans with diabetes. The most dramatic hypertriglyceridemia is found in patients who develop diabetes and have an underlying defect in LpL, e.g., heterozygous LpL deficiency (33). Conversely, we hypothesize that patients with greater LpL activity have less diabetes-induced hypertriglyceridemia.

It should be noted that both on chow and on the WTD, diabetes did not reduce HDL levels in mice. Low HDL is a common lipoprotein abnormality in diabetic humans, and we presume that the lack of cholesteryl ester transfer protein in the mice prevented the increase in VLDL from reducing HDL. Elevated VLDL permits more HDL cholesteryl ester to exchange with VLDL triglyceride. In a previous report (21), despite lower triglyceride and greater lipolysis, the MCK-LpL mice had lower, rather than higher, HDL cholesterol. In the HuBTg mice, we found that the MCK-LpL transgene was associated with lower HDL. We also did not find an HDL increase in the diabetic mice that expressed the MCK-LpL transgene. The reasons for these effects of muscle LpL are unclear and, at first glance, appear to be in conflict with the well-established role of LpL to increase HDL by lipolysis of triglyceride and by transfer of excess surface lipid to HDL. More recently, it has become apparent that LpL has non-enzymatic actions. In this context, LpL, as well as hepatic lipase (34, 35), can promote selective uptake of HDL lipid (36). Perhaps in mice containing the MCK-LpL transgene, this action, rather than LpL-mediated lipolysis predominates as the mice have very low levels of VLDL and LpL is not limiting.

We purposely created severely diabetic mice both to exacerbate any diabetes-induced lipoprotein changes and to determine whether the severely hyperglycemic mice had more atherosclerosis. The differences in atherosclerosis between diabetic and non-diabetic mice in our study were small, but the most consistent result was a failure of diabetes to increase atherogenesis. This was observed in both male and female mice. Although at first it may appear surprising that we did not find such an effect, our results are consistent with others in the literature. Diabetic LDL receptor knockout mice do not have more atherosclerosis than control mice (37). Except for a small increase in lesions in BALB/c mice, most non-transgenic strains of mice do not have diabetes-induced atherogenesis (16). Recently, Park et al. (38) found that diabetes increased lesion size in diabetic mice deficient in apolipoprotein E, an effect that was inhibited by infusion of soluble fragments of the receptor for advanced glycosylation end products (RAGE). In these mice the diabetes markedly increased circulating cholesterol levels. Therefore, the secondary hyperlipidemia rather than effects of the diabetes itself might have been the primary reason for the increased atherosclerosis. Alternatively, the effects of diabetes may be model specific.

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These studies in mice need to be compared with those in other animals. Cholesterol-fed rabbits develop severe hypertriglyceridemia when made diabetic (39). These large triglyceride-rich lipoproteins are unable to enter the arterial wall (40). Perhaps for this reason, diabetes unexpectedly decreases atherosclerosis in rabbits (41). Recently, diabetes in swine was shown to markedly increase atherosclerosis (42). Taken together, it appears that diabetes-related increases in atherosclerosis are species specific. As all diabetic animals have hyperglycemia, differences in the response to glucose or advanced glycosylation end products may vary. Alternatively, secondary changes in blood pressure or plasma lipids that accompany diabetes may be more important modulators of atherogenesis than glucose. The decreases in macrovascular disease in diabetic patients who received anti-hypertensive (43) and cholesterol-lowering therapy (44) support this conclusion.

In summary, lipids and lipoprotein profiles were studied in HuBTg mice with STZ-induced diabetes and in similar mice overexpressing LpL in the muscle. Severe diabetes produced a modest increase in VLDL cholesterol and triglyceride; LpL overexpression almost totally obliterated the lipoprotein alterations due to diabetes. The lipoprotein changes that occurred were similar to those found in humans with diabetes, except that HDL did not decrease in the mice. The increase in VLDL was not associated with increased triglyceride production, and appeared to result from a reduction in VLDL catabolism. LpL activity was not reduced in the diabetic mice. The lower LpL activity in humans and its alteration during diabetes may be the reason why human diabetes leads to a more severe dyslipidemia. Diabetes did not increase atherosclerosis in these mice. From our data, we postulate that exacerbation of the dyslipidemia, rather than just hyperglycemia, is needed to reproduce diabetes-related macrovascular disease in mice.

In this light, our studies of atherosclerosis in these diabetic mice can be interpreted in either of two ways. *1*) Mice are a "bad" model of human diabetic macrovascular disease. If this is true, it suggests that there are some basic genetic differences in the mouse that protect it from the seemingly deleterious effects of hyperglycemia on large blood vessels. Mouse atherosclerosis could also be fundamentally different than human disease; despite nearly a decade of experimental work, the mouse might be a flawed model. *2*) Mice are an appropriate model; however, the diabetes-mediated alterations that are responsible for atherosclerosis exacerbation are missing in HuBTg mice. This could include a number of glucose-sensitive processes, one of which is dyslipoproteinemia. To test this, we have begun follow-up studies in which hypertriglyceridemia and HDL reduction are a prominent feature of diabetes-mediated metabolic changes. If diabetes increases atherosclerosis in these new models, it would prove that diabetes-induced lipoprotein changes are required for atherosclerosis exacerbation.

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