Effect of fasting on the composition of plasma lipoproteins in cholesterol-fed diabetic rabbits

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Abstract Cholesterol-fat feeding is associated with unusual alterations in the composition of plasma lipoproteins in alloxan-diabetic rabbits. In the present study plasma lipoprotein lipid and apoprotein composition was studied before and after 48 hr of fasting in cholesterol-fed diabetic and control rabbits in order to further characterize these alterations. Compared with control rabbits, the diabetic rabbits had similar plasma cholesterol levels, but 100-fold higher triglyceride levels prior to fasting. These plasma lipids were distributed mainly to large, $S_f > 400$ plasma lipoproteins in the diabetic rabbits, and to β-VLDL in control rabbits. $S_f > 400$ lipoproteins, VLDL, IDL, LDL, and HDL from diabetic rabbits had triglyceride as the predominant lipoprotein core lipid. $S_f > 400$ lipoproteins and VLDL from diabetic rabbits had lesser amount of apoprotein E, and greater amounts of apoproteins A-I, A-IV, and B-48 as percent of total apoprotein mass in comparison with control rabbits. Fasting reduced plasma triglyceride levels by 55% in diabetic rabbits. $S_f > 400$ lipoprotein and VLDL triglyceride content decreased but remained a major core lipid. Fasting eliminated apoproteins A-I and A-IV from $S_f > 400$ lipoproteins and VLDL, but had no significant effect on apoB-48 content. Insulin treatment of the diabetic rabbits reduced plasma triglyceride by approximately 90% resulting in cholesteryl ester-rich particles reassembling β-VLDL both in the $S_f > 400$ lipoprotein and VLDL fractions. These results indicate that the alterations in plasma lipoproteins in cholesterol-fed diabetic rabbits result from the presence in the d < 1.006 g/ml plasma lipoprotein class of partially metabolized, intestinally derived particles. —Van Sickle, W. A., P. Brecher, A. Aspen, and A. V. Chobanian. Effect of fasting on the composition of plasma lipoproteins in cholesterol-fed diabetic rabbits. *J. Lipid Res.* 1985. **26:** 442–450.

Supplementary key words alloxan diabetes • apoproteins • effects of fasting

Early studies of the relationship between diabetes and diet-induced atherosclerosis in rabbits showed that alloxan-diabetic, cholesterol-fed rabbits develop less aortic atherosclerosis than non-diabetic rabbits (1), and that insulin treatment reverses this resistance to atherosclerosis (2). In response to cholesterol feeding, the untreated diabetic rabbits develop degrees of hypertriglyceridemia and hypercholesterolemia that are much greater than in non-diabetic rabbits fed similar amounts of dietary fat, and there is an unusual accumulation of $S_f > 80$ lipoproteins in their plasma (3, 4).

In a recent study we re-examined this animal model, and confirmed the results of the earlier studies showing protection towards cholesterol-induced atherosclerosis, hypertriglyceridemia, and an accumulation of d < 1.019 g/ml plasma lipoproteins (4). We examined the chemical and physical properties of the d < 1.019 g/ml plasma lipoprotein fraction and found that most of the plasma triglyceride and cholesteryl esters coexisted in the core of a relatively homogenous population of large particles. These particles did not stimulate cholesteryl ester formation when incubated with mouse peritoneal macrophages, an effect that was observed when β-VLDL from the cholesterol-fed, non-diabetic rabbits were added. These results suggested that the resistance to diet-induced atherosclerosis in the diabetic rabbit may be due to the properties of these unusual particles.

In the present study we have further characterized the lipid and apoproteins in individual lipoprotein classes of the diabetic cholesterol-fed rabbits, in both the ad lib fed and 48-hr fasted state, and have made comparisons with lipoproteins from cholesterol-fed non-diabetic as well as insulin-treated diabetic rabbits.

METHODS AND MATERIALS

Animals

Male, New Zealand white rabbits, weighing between 2.4 and 3.1 kg, were purchased from Pine Acres Farm, Brattleboro, VT. They were housed individually and fed Purina Rabbit Chow (Ralston Purina Co., St. Louis, MO) ad lib. Randomly selected rabbits were treated with alloxan monohydrate (Sigma Chemical Co., St. Louis, MO). Each animal received a single 150 mg/kg dose infused at 11.0 mg/kg per min I.V. to induce diabetes (5).

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; PAGE, polyacrylamide gel electrophoresis.
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The alloxan-treated rabbits were given a 6% solution of dextrose in tap water for 24 hr after treatment. Rabbits with plasma glucose levels of between 270 and 560 mg/dl 48 hr after treatment, and after a 6-hr fast, were selected for study. Two weeks after treatment, the diabetic rabbits' chow was replaced with a diet containing 0.5% cholesterol and 7% corn oil. They were kept on this diet for 10 to 14 days. Untreated non-diabetic rabbits were fed a diet containing 1.5% cholesterol and 6% corn oil for 12 to 16 days in order to more closely match their plasma cholesterol levels with those of the diabetic rabbits. At the end of the feeding period blood was collected from a lateral vein of an ear into tubes containing K_2EDTA and sodium azide. The animals were then fasted for 48 hr and bled again.

A separate group of cholesterol-fed diabetic rabbits was treated with protamine-zinc insulin (1.0 U/kg per day, subcutaneously as a single dose). These animals were fed the same diet as the other diabetic rabbits for 7 days prior to, and for 10 days following, the initiation of insulin treatment. They were fasted overnight prior to collecting blood for plasma lipid and lipoprotein analysis before and after the interval of insulin treatment. The animals were bled 2.5 hr after the last insulin injection.

Lipoprotein isolation

Lipoprotein isolation was begun immediately after blood was drawn. Blood was kept on ice and centrifuged at 4°C to obtain plasma. Three to 5 ml of plasma was layered under a 0.195 M NaCl solution containing 0.02% NaN_3 and 0.01% K_2EDTA (d 1.006 g/ml), and centrifuged at 12°C in a Beckman L2-65B centrifuge using a 50 Ti rotor and thin-walled polyallomer tubes. $S_f > 400$ lipoproteins were isolated using the nomogram of Dole and Hamlin (6). The centrifuge tubes were sliced 5.5 cm from the bottom after centrifugation for 3.9×10^6 g-min (avg) to float the $S_f > 400$ lipoproteins. VLDL, IDL, LDL, and HDL were isolated in sequence from the $S_f > 400$ infranatant at densities equal to 1.006, 1.019, 1.063, and 1.21 g/ml, respectively. VLDL, IDL, and LDL were isolated by centrifugation for 1.2×10^8 g-min (avg). HDL was isolated after centrifugation for 2.5×10^8 g-min (avg). The $S_f > 400$ plasma fraction was adjusted to a density of approximately 1.06 g/ml by the addition of NaBr, layered under the above d 1.006 g/ml solution, and re-isolated as above in order to minimize albumin contamination. Lipoprotein fractions were delipidated with 40 volumes of ethanol-diethyl ether 3:2 (vol/vol) at –15°C overnight, then centrifuged at 2000 rpm for 30 min at 4°C. The pellet and the apoproteins were separated and identified using either 4% or 10% polyacrylamide slab gels (PAGE) as described previously (4). Quantitative estimates of differences in aproprotein content of $S_f > 400$

and VLDL lipoproteins between diabetic and non-diabetic rabbits were obtained by scanning 10% polyacrylamide gels with a densitometer at 560 nm (Transidyne model 2955; Transidyne General Corp., Ann Arbor, MI). Plasma lipoproteins and isolated lipoproteins were separated on 1% agarose gels using a Corning electrophoresis system and following the manufacturer's instructions (Corning Medical, Corning Glass Works, Medfield, MA).

Gel filtration

The $S_f > 400$ and VLDL lipoproteins were chromatographed on 2.6×60 cm ascending columns of either 1% or 2% agarose (Bio-Gel A-150m or A-50m, respectively, both 100–200 mesh; Bio-Rad Laboratories, Richmond, CA) in order to estimate their relative sizes (7). Rat chylomicrons isolated from intestinal lymph were generously supplied by Dr. T. Redgrave. One-ml samples were applied to the columns, and 4.0-ml fractions were collected at 4°C using 0.15 M NaCl, 0.01 M sodium-phosphate buffer, pH 7.2, containing 0.01% NaN_3 as the eluent. The flow rate was 17 ml/hr.

Chemical assays

Plasma glucose was measured by the glucose oxidase method (8) after removing lipids and proteins with $Ba(OH)_2$-$ZnSO_4$ precipitation (9). Total plasma and lipoprotein cholesterol was measured with the use of a kit (Boehringer-Mannheim), and esterified cholesterol was measured as the difference between free and total cholesterol. Plasma and lipoprotein triglyceride was determined by an enzymatic assay for glycerol (10) after saponification of the sample in alcoholic KOH. Phospholipid phosphorus was measured as described by Barlett (11) with a factor of 25 used to convert phosphorus to phospholipid. Protein was measured by the method of Lowry et al. (12), with chloroform extraction of samples and standards to remove turbidity. Lipoprotein lecithin and sphingomyelin content was determined from lipid extracts (13) which were evaporated to dryness under nitrogen and redissolved in toluene. Samples were analyzed by thin-layer chromatography using chloroform methanol ammonia 60:34:3 as the developing solvent. The phospholipids were quantitated by scanning densitometry after spraying the plates with 50% H_2SO_4 and charring on a hot plate.

Statistics

Data were summarized by calculating means and standard errors, except where noted. The Mann-Whitney U-test was used to test for differences between diabetic and control groups, and paired t-tests were used to test for significant effects of fasting.

RESULTS

Plasma glucose, lipids, and lipoproteins

Plasma glucose levels in the diabetic rabbits used in this study were 475 ± 22 mg/dl ($\bar{x} \pm$ SE) after the 10–14-day interval of cholesterol feeding. Fasting for 48 hr reduced their plasma glucose levels to 409 ± 20 mg/dl. The corresponding values (fed vs. fasted) for the non-diabetic control animals were 120 ± 4 and 97 ± 2 mg/dl, respectively. Body weights of diabetic animals were 2.83 ± 0.10 kg, and 3.00 ± 0.13 kg for control animals midway during the course of cholesterol feeding.

Plasma from ad lib fed diabetic rabbits showed a diffuse and irregular electrophoretic profile on 1% agarose gels (**Fig. 1**). The isolated $S_f > 400$ lipoproteins appeared to be responsible for this because they, but no other lipoprotein, had a similar pattern. The VLDL from diabetic rabbits had pre-β mobility, unlike those from control rabbits which had a mobility closer to that of LDL. The mobility of LDL from diabetic and control animals was identical on agarose gels.

Plasma cholesterol and triglyceride levels and the distribution of these lipids among the major lipoprotein classes in the animals studied are shown in **Table 1**. In the ad lib fed state, the diabetic rabbits had plasma cholesterol levels that were similar to those in control rabbits, but their plasma triglyceride levels were 100-fold higher. An average of 76% of the plasma cholesterol was recovered in d < 1.006 g/ml lipoproteins ($S_f > 400$ and VLDL) from diabetic, and 61% from control rabbits. The diabetic rabbits had significantly more $S_f > 400$ lipoprotein cholesterol than the non-diabetic rabbits. The amounts of IDL, LDL, and HDL cholesterol were significantly less in diabetic compared with control rabbits. With the exception of HDL, all lipoprotein classes from diabetic rabbits carried more triglyceride than was found in lipoproteins
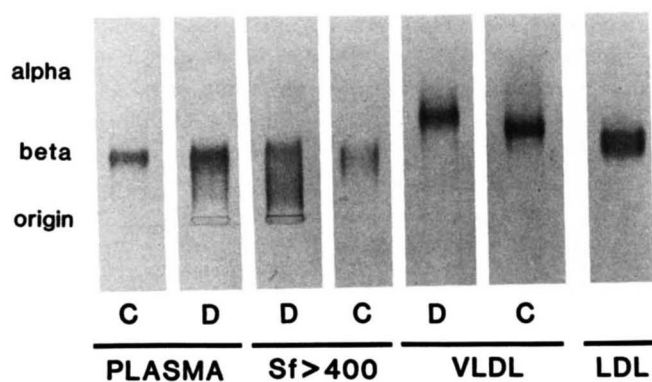
from control rabbits. The molar ratios of triglyceride/cholesteryl esters in the $S_f > 400$ and VLDL fractions in the diabetic animals were 2.0 ± 0.3 and 1.7 ± 0.3 ($\bar{x} \pm$ SD), respectively. Plasma cholesterol levels remained unchanged after 48 hr of fasting in the diabetic rabbits. There was a slight decrease in $S_f > 400$ cholesterol, while VLDL, IDL, LDL, and HDL cholesterol levels tended to increase. Plasma triglyceride levels decreased by an average of 55% ($P < 0.01$, paired t-test) upon fasting in the diabetic rabbits. This reduction was confined to the $S_f > 400$ lipoprotein fraction, which lost 60% of its triglyceride mass. The molar ratio of triglyceride/cholesteryl ester decreased upon fasting to less than 1.0 in both $S_f > 400$ lipoprotein and VLDL fractions.

Plasma cholesterol decreased by 216 ± 56 mg/dl ($P < 0.05$, paired t-test) upon fasting in control rabbits (Table 1). This reduction resulted from decreases in IDL and LDL cholesterol. $S_f > 400$ cholesterol tended to decrease while VLDL cholesterol increased, resulting in no change in d < 1.006 g/ml lipoprotein cholesterol in these rabbits. Plasma and lipoprotein triglycerides increased slightly upon fasting in control rabbits.

The lipid composition of individual lipoprotein classes from both groups of rabbits in the fed and fasted states is shown in **Table 2**. In the fed state, all lipoproteins from diabetic rabbits tended to have lower percentages of free cholesterol and higher percentages of phospholipid compared with the control rabbit. All of their lipoproteins were enriched in triglyceride relative to cholesteryl esters. In control rabbits, triglyceride was a minor component of the lipoprotein lipid, and the particles exhibited the cholesteryl ester enrichment characteristic of lipoproteins from cholesterol-fed rabbits (14–16). Fasting reduced the triglyceride content of lipoproteins from diabetic rabbits, but it still remained a major component. There were slight but consistent increases in lipoprotein triglyceride content in control animals.

Differences in lipid composition between control and diabetic animals were also observed when the distribution of phospholipid classes in d < 1.006 g/ml ($S_f > 400$ and VLDL) lipoprotein fractions were analyzed. **Table 3** shows a major difference in the relative amounts of lecithin and sphingomyelin present. The lecithin/sphingomyelin ratio of particles from fed diabetic rabbits was about 20:1, whereas in control animals this ratio was 5:1. Fasting for 48 hr led to a marked change in the ratio in diabetic animals (11:1) but fasting did not alter the ratio in the cholesterol-fed controls.

The $S_f > 400$ and VLDL lipoproteins were also characterized by gel filtration to estimate the relative sizes of the lipoprotein particles contained in these fractions. Figs. 2 and 3 show representative data for diabetic animals. Using Bio-Gel A-50m, the $S_f > 400$ particles appeared heterogenous with the main fraction eluting at the void volume (**Fig. 2A**). VLDL was included in the

Fig. 1. One percent agarose electrophoretograms of plasma, $S_f > 400$, and VLDL from cholesterol-fed diabetic (D) and control (C) rabbits. The animals were bled in the ad lib fed state. The LDL was obtained from a cholesterol-fed control rabbit.

TABLE 1.   Concentration of cholesterol and triglyceride in plasma and lipoproteins in cholesterol-fed diabetic and control rabbits in the fed state and after a 48-hr fast

	Plasma	$S_f > 400$	VLDL	IDl	LDL	HDL
	Cholesterol (mg/dl)[a]					
Fed diabetic	1837 ± 37	1209 ± 27[b]	188 ± 37	20 ± 7[b]	26 ± 8[b]	5 ± 2[b]
Fed control	1485 ± 372	518 ± 220	388 ± 110	230 ± 33	167 ± 45	18 ± 3
Fasted diabetic	1771 ± 98	1032 ± 75[b]	351 ± 84	45 ± 16[b]	66 ± 27[b]	15 ± 1[c]
Fasted control	1269 ± 365[c]	305 ± 130	503 ± 177	148 ± 20[c]	99 ± 21[c]	16 ± 3
	Triglyceride (mg/dl)					
Fed diabetic	5543 ± 557[b]	4284 ± 495[b]	485 ± 114[b]	34 ± 11[b]	39 ± 15[b]	9 ± 5
Fed control	58 ± 25	23 ± 13	11 ± 7	10 ± 4	7 ± 2	5 ± 2
Fasted diabetic	2505 ± 531[b,d]	1695 ± 470[b,d]	337 ± 67[b]	42 ± 10	46 ± 18	10 ± 6
Fasted control	163 ± 49	33 ± 13	47 ± 17	14 ± 4	16 ± 7	6 ± 1

[a]Values are mean ± SE; n = 4 in each group.
[b]$P < 0.05$ compared to control rabbits (Mann-Whitney U-test).
[c]$P < 0.05$ compared to corresponding fed animal (paired t-tests).
[d]$P < 0.01$ compared to corresponding fed animal (paired t-tests).

column and appeared as a broad peak separated from S_f > 400 particles (Fig. 2B). Similar elution profiles were obtained from fed and fasted animals. The S_f > 400 fraction, as well as rat chylomicrons, was also analyzed using Bio-Gel A-150m (**Fig. 3**). The S_f > 400 fraction was included in the column, and its cholesterol eluted as a symmetrical peak. In contrast, chylomicrons obtained from the thoracic duct of a corn oil-fed rat were mostly absorbed into the column and a small amount of material that did elute appeared at the void volume. Thus, the S_f > 400 fraction from diabetic animals was most likely smaller than intact chylomicrons and larger than VLDL.

Apoprotein composition

The apoproteins of lipoproteins from fed and fasted animals were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Using 10% gels, the S_f > 400 and VLDL lipoproteins from fed diabetic rabbits were found to contain apoproteins with estimated molecular weights of 26,700, 42,800, and 34,900, corresponding to apoproteins A-I, A-IV, and E, respectively (**Fig. 4**). High molecular weight apoproteins were found near the origin (apoBs), and apoproteins of molecular weights less than 20,000 were found near the tracking dye. In contrast,

TABLE 2.   Percent lipid composition of lipoproteins from fed and fasted diabetic and control rabbits[a]

Animals	Lipid	Lipoproteins				
		$S_f > 400$	VLDL	IDL	LDL	HDL
Fed						
Diabetic	C	5.3 ± 1.0	6.8 ± 1.5	9.0 ± 1.4	9.5 ± 1.3	6.0 ± 0.0
	CE	23.3 ± 4.1	23.8 ± 4.6	23.8 ± 6.0	24.8 ± 3.3	19.0 ± 7.0
	TG	60.8 ± 7.5	50.8 ± 7.2	40.0 ± 8.1	34.5 ± 6.5	34.0 ± 7.1
	PL	11.5 ± 1.3	19.0 ± 2.7	26.5 ± 2.6	31.5 ± 4.4	41.0 ± 0.0
Control	C	6.8 ± 2.2	10.0 ± 2.8	12.8 ± 3.7	13.3 ± 3.0	7.8 ± 1.0
	CE	80.8 ± 2.2	72.8 ± 2.4	64.3 ± 4.7	59.0 ± 4.7	46.0 ± 6.8
	TG	2.0 ± 2.8	1.0 ± 1.0	2.3 ± 1.5	2.8 ± 1.3	9.5 ± 6.8
	PL	10.3 ± 1.0	16.5 ± 1.3	20.5 ± 2.1	25.8 ± 3.0	36.8 ± 2.9
Fasted						
Diabetic	C	6.8 ± 1.0	7.8 ± 2.9	8.5 ± 2.4	8.8 ± 1.5	5.0 ± 1.4
	CE	40.0 ± 8.1	42.8 ± 7.1	37.5 ± 6.8	40.5 ± 5.6	28.5 ± 6.4
	TG	41.5 ± 9.9	31.8 ± 8.4	33.3 ± 7.3	23.5 ± 5.9	27.5 ± 10.6
	PL	11.3 ± 1.9	18.0 ± 1.2	21.0 ± 1.2	27.5 ± 2.6	35.0 ± 19.8
Control	C	8.5 ± 2.9	10.3 ± 3.1	12.5 ± 2.9	15.0 ± 4.3	7.8 ± 1.0
	CE	73.5 ± 5.3	65.3 ± 8.3	59.5 ± 7.6	59.0 ± 7.3	38.3 ± 9.9
	TG	5.0 ± 4.5	5.3 ± 3.8	4.8 ± 3.1	6.5 ± 5.2	12.8 ± 8.2
	PL	13.3 ± 2.9	19.3 ± 3.4	23.3 ± 3.9	20.3 ± 8.6	41.0 ± 6.8

C, unesterified cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid.
[a]Values are mean ± SD; n = 4 in each group.

TABLE 3. Lecithin-sphingomyelin ratios in d < 1.006 g/ml lipoproteins from cholesterol-fed diabetic and control rabbits in the fed state and after a 48-hr fast[a]

	Fed Diabetic	Fasted Diabetic	Fed Control	Fasted Control
$S_f > 400$	19.5 ± 1.9	10.6 ± 1.0[b]	4.6 ± 1.5	4.1 ± 0.6
VLDL	19.4 ± 3.2	10.7 ± 1.7[b]	5.5 ± 0.8	5.7 ± 1.4

[a]Values are the mean ± SE from four diabetic and three control rabbits.
[b]$P < 0.05$ compared with fed values (paired t-test).

these lipoproteins from control animals contained predominantly apoBs and E; there were only trace amounts of apoA-I or apoA-IV. These differences between diabetic and control rabbits were quantified using scanning densitometry. In **Table 4** it can be seen that the percentages of the A apoproteins in d < 1.006 g/ml lipoproteins of fed

diabetic rabbits were higher than those in controls. It is also apparent that there was less apoE, and more of the low molecular weight species of apoB (B-48) in these lipoproteins from diabetic animals in comparison with the control animals. Fig. 4 also shows the apoprotein profiles in d < 1.006 g/ml lipoproteins from a representative fasted diabetic rabbit. These lipoproteins, like those in control animals, were devoid of apoA-I and apoA-IV. There appeared to be more apoE in d < 1.006 g/ml lipoproteins from fasted diabetic than in fed diabetic rabbits.

Fig. 5 shows the electrophoretic pattern on 4% SDS-polyacrylamide gels of the high molecular weight B apoproteins in d < 1.006 g/ml lipoproteins from both fed and fasted diabetic rabbits, and in VLDL from a fed control rabbit. The B-48 protein was a major apoprotein in S_f > 400 lipoproteins and VLDL from fed and fasted dia-
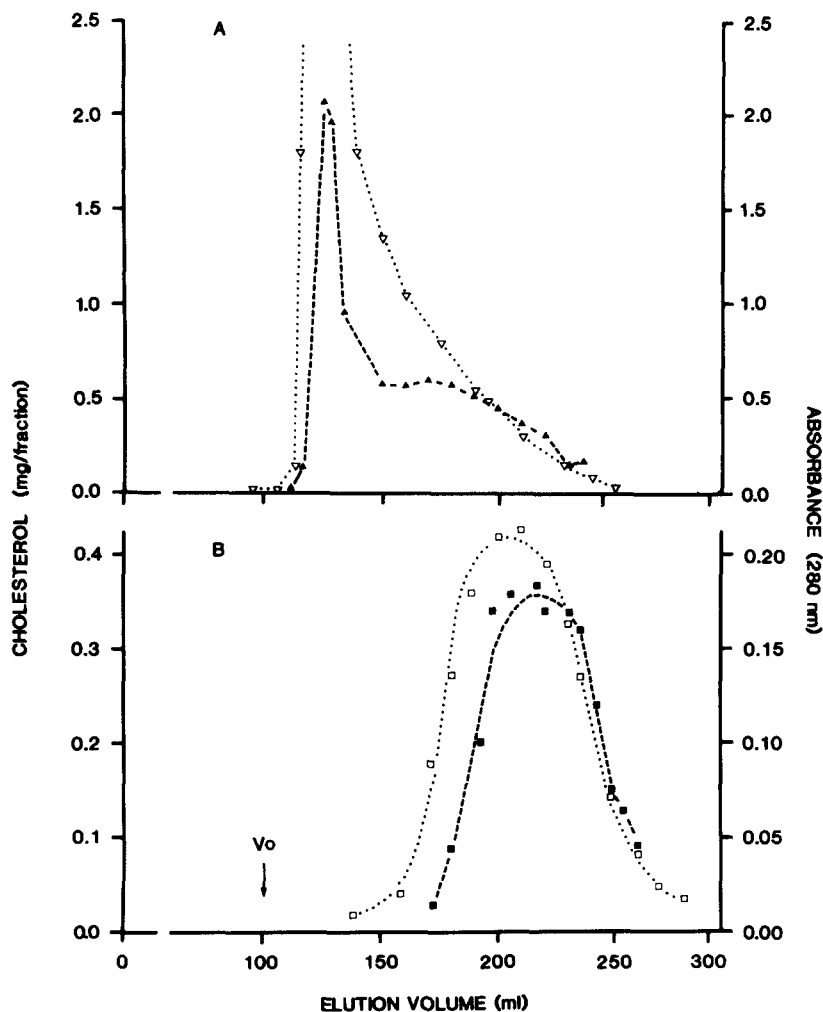

Fig. 2. Elution patterns of S_f > 400 (A) and VLDL (B) lipoproteins from a cholesterol-fed diabetic rabbit on a column of 2% agarose. Lipoprotein cholesterol (▲- - -▲, S_f > 400; ■- - -■, VLDL) and absorbance (△· · ·△, S_f > 400; □· · ·□, VLDL) were determined on individual fractions. Each plasma lipoprotein fraction was chromatographed separately. Vo indicates void volume of the column.
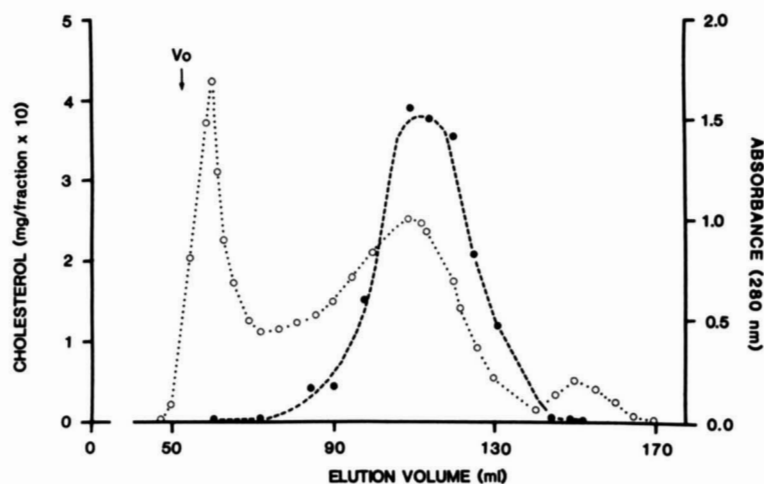
Fig. 3. Elution pattern of $S_f > 400$ lipoprotein cholesterol ($\bullet$---$\bullet$) and absorbance ($\circ\cdots\circ$) from a cholesterol-fed diabetic rabbit on a column of 1% agarose. Vo indicates void volume of the column.

betic rabbits. ApoB-100 was present in both $S_f > 400$ lipoproteins and VLDL, and in both fed and fasted diabetic rabbits. ApoB-100 was the predominate species of apoB in VLDL from control rabbits; apoB-48 was present in only trace amounts.

Apoprotein B-100 was a major species in IDL and LDL from both diabetic and control animals (**Fig. 6**). The IDL from control animals contained apoE in addition to B-100, while IDL from diabetic rabbits lacked apoE. Apoprotein A-I was the dominant apoprotein in HDL from both types of animals. Fasting did not affect the apoprotein profiles of IDL, LDL, and HDL in either diabetic or control rabbits, nor did it affect the apoprotein profile of $S_f > 400$ lipoproteins or VLDL from control rabbits (data not shown).

Insulin treatment

The effects of insulin treatment for 10 days on plasma glucose, lipids, and on the composition of $S_f > 400$ lipoproteins and VLDL from cholesterol-fed diabetic rabbits are given in **Table 5** and **Table 6**. Plasma glucose levels were reduced by insulin therapy, with normal values obtained 2.5 hr after insulin injection. Plasma cholesterol levels were unchanged. Plasma triglyceride levels in all of the diabetic rabbits were markedly reduced, with the median triglyceride level being lowered by over 90%. This effect was clearly more pronounced than the effect of a 48-hr fast. The $S_f > 400$ lipoproteins and VLDL isolated from the plasma of these diabetic rabbits were enriched in cholesteryl ester relative to triglyceride, although the triglyceride content was greater than that from the cholesterol-fed control rabbits summarized above.

The apoproteins of $d < 1.006$ g/ml lipoproteins from insulin-treated, cholesterol-fed diabetic rabbits are shown in **Fig. 7**. In contrast to untreated diabetic rabbits, no

trace of either apoA-I or apoA-IV was found, and apoB-48 appeared to be a minor component. In general, the apoprotein and lipid composition of these particles was similar to that from cholesterol-fed control animals.

DISCUSSION

Plasma cholesterol levels in the diabetic rabbits were comparable to those of the non-diabetic animals despite the fact that they consumed a diet with only one-third the cholesterol content. A 48-hr fast had little effect on plasma cholesterol levels in the diabetic animals, and most of the
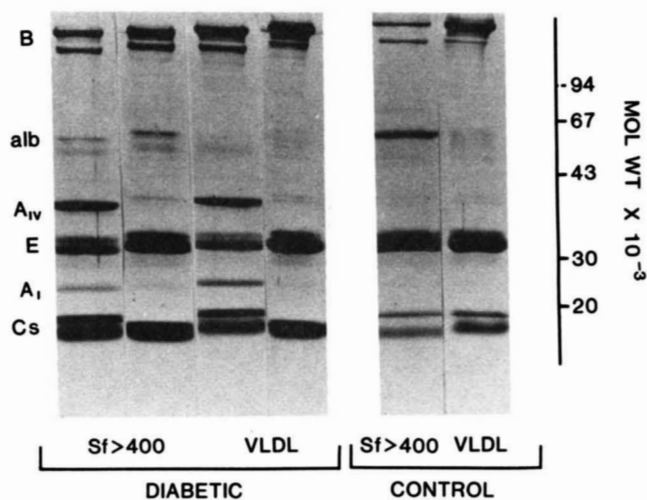

Fig. 4. SDS-polyacrylamide gel (10%) electrophoretograms of $S_f > 400$ and VLDL apoproteins from cholesterol-fed diabetic and control rabbits. The lipoproteins from the control rabbit were obtained during ad lib feeding. For the diabetic rabbit, the left member of each pair of $S_f > 400$ and VLDL lipoproteins was obtained during ad lib feeding, and the right member after a 48-hr fast; alb, albumin.

TABLE 4. Percent distribution of high and intermediate weight apoproteins in d < 1.006 g/ml lipoproteins from cholesterol-fed diabetic and control rabbits[a]

	Apoproteins				
	B-100	B-48	A-IV	E	A-I
$S_f > 400$					
Diabetic	18 ± 3	11 ± 1	12 ± 7	56 ± 9	3 ± 1
Control	12 ± 3	5 ± 2	<2	82 ± 1	<2
VLDL					
Diabetic	42 ± 3	9 ± 1	9 ± 4	35 ± 4	4 ± 2
Control	32 ± 6	3 ± 1	<2	64 ± 7	<2

The C apoproteins were not scanned due to poor resolution from the tracking dye.

[a]Values are mean ± SE from four diabetic and three control rabbits.

cholesterol remained in the $S_f > 400$ plasma lipoprotein fraction. Plasma triglyceride levels were reduced by over 55% as a result of fasting, and could be accounted for almost entirely by a reduction in the triglyceride content of the $S_f > 400$ fraction. This suggests that these animals retain the same ability to clear triglycerides from the circulation.

Apoproteins A-I and A-IV were isolated from both the $S_f > 400$ and VLDL fraction from fed diabetic rabbits, but were not found in either of these fractions from control animals. Both apoA-I and apoA-IV are known to be secreted with nascent chylomicrons (17), and are normally transferred to HDL or the d > 1.21 g/ml fraction of plasma during the formation of chylomicron remnants (18, 19). The absence of apoA-I and A-IV from d < 1.006 g/ml lipoprotein from control rabbits presumably reflects the rapid clearance of dietary triglyceride and the lipoproteins that carry it to the circulation (20). The presence of these apoproteins associated with triglyceride-rich lipoproteins in the diabetic rabbits suggests that the metabo-

lism of chylomicrons and VLDL was defective in these animals.

Both apoB-100 and apoB-48 were present in $S_f > 400$ and VLDL fractions from the diabetic rabbits, and the relative amounts of apoB-48 in these fractions were markedly greater than was observed in the control rabbits. ApoB-48 is synthesized in the intestines of several species (21), and its presence in plasma is indicative of chylomicrons or chylomicron remnants. ApoB-100 is generally considered to be associated with particles of hepatic origin. Kane et al. (22) fractionated d < 1.006 g/ml lipoproteins from patients with Type III hyperlipoproteinemia into β- and preβ-migrating particles and found decreasing ratios of B-100 and B-48 as the diameters of the β-migrating particles increased. Similar observations were made by Fainaru et al. (23) on d < 1.006 g/ml lipoproteins from cholesterol-fed dogs, where it was found that β-migrating particles consisted of two populations that differed in several physical, chemical, and metabolic respects: the larger particles, which were diminished in number by 48 hr of fasting, had approximately equal amounts of B-100 and B-48, whereas the smaller particles contained predominantly apoB-100. The larger particles, with the higher content of apoB-48, were rapidly cleared from the circulation. These authors concluded that the d < 1.006 g/ml plasma lipoprotein fraction was composed of chylomicron remnants as well as particles of hepatic origin.

The plasma clearance of apoB-48 in triglyceride-rich lipoprotein has recently been shown to be diminished in patients with lipoprotein lipase deficiency (24). This is consistent with the ability of this enzyme to mediate the clearance of chylomicrons (25), and of apoB-48 being a constituent of these lipoproteins. Slower clearance rates of apoB-48-containing particles have also been observed in severely hypertriglyceridemic human diabetics and in
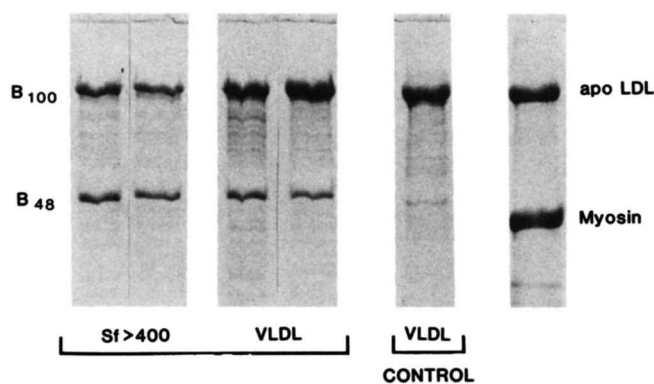

Fig. 5. SDS-polyacrylamide gel (4%) electrophoretograms of the same lipoprotein apoproteins shown in Fig. 4. The left member of each pair of $S_f > 400$ and VLDL apoproteins from the diabetic rabbit, as well as the VLDL from the control rabbit, was obtained during ad lib feeding, and the right member after a 48-hr fast.
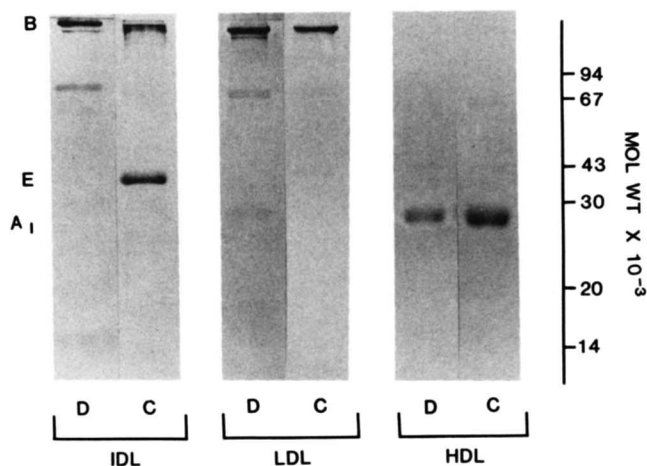

Fig. 6. SDS-polyacrylamide gel (10%) electrophoretograms of IDL, LDL, and HDL apoproteins from cholesterol-fed diabetic (D) and control (C) rabbits. Lipoproteins were obtained during ad lib feeding.

TABLE 5. Effect of insulin on plasma glucose, cholesterol, and triglyceride concentration in cholesterol-fed diabetic rabbits

Plasma	Glucose[a]	Cholesterol[a]	Triglycerides[b]
Before PZI[c]	418 ± 21	1847 ± 272	8051 (2257–20128)
After PZI	74 ± 15	2016 ± 524	774 (242–6601)

[a]Mean ± SE, mg/dl, n = 5.
[b]Median and range, mg/dl.
[c]Protamine-zinc insulin.

patients with Type V hyperlipoproteinemia (26).

ApoB-48 was a prominent component of the d < 1.006 g/ml plasma lipoproteins from the diabetic rabbits in the present study. Despite extensive lypolysis, and modification of lipoprotein surface constituents including a gain in apoE, loss of apoA-I and A-IV, and reduced lecithin/sphingomyelin ratio after a 48-hr interval of fasting, apoB-48 remained a predominant apoprotein in this plasma fraction. From these data, it is reasonable to conclude that the ability of the diabetic animals to degrade chylomicrons to remnants, and to clear incompletely formed remnants from the circulation, was severely limited.

ApoB-100 was clearly the major species of apoB in the d < 1.006 g/ml plasma lipoproteins from the cholesterol-fed control rabbits. Earlier studies suggested that this β-VLDL fraction in rabbits arose from intestinally derived particles (27); but if apoB-100 is the sole species of apoB secreted by the liver in rabbits, as it is in other animals (21), then the β-VLDL in our non-diabetic control rabbits may have originated as hepatic particles. ApoB-100, as well as B-48, was present in d < 1.006 g/ml lipoproteins in the diabetic rabbits, suggesting that both hepatic and intestinally derived particles were present in this lipoprotein fraction. We have previously shown that this lipoprotein fraction consists of a population of particles that contains triglycerides and cholesteryl esters together within the core of the same particle (4). Presumably, the triglycerides originally contained in intestinally derived particles equilibrated with hepatic particles through the
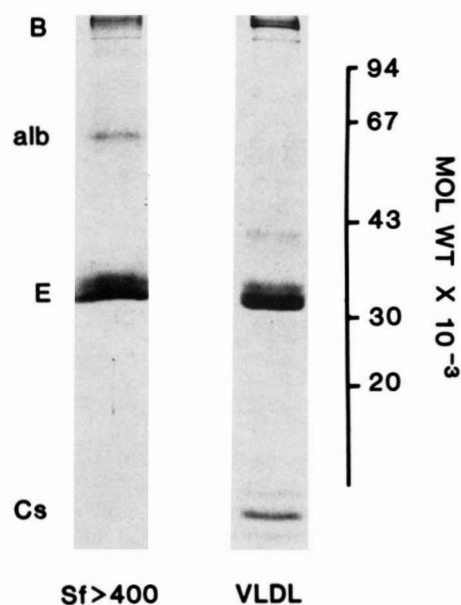

Fig. 7. SDS-polyacrylamide gel (10%) electrophoretograms of S_f > 400 and VLDL apoproteins from an insulin-treated, cholesterol-fed diabetic rabbit.

action of triglyceride and cholesteryl esters exchange proteins (28) in the diabetic rabbits. Because of the impaired clearance of dietary triglycerides in the diabetic animal, there was sufficient time for such exchange to occur. In the cholesterol-fed control rabbit, lypolysis of dietary triglyceride is rapid and remnant particles are cleared from circulation (29). The action of the lipid exchange and transfer proteins, coupled with reduced triglyceride clearance, may also explain the high triglyceride content of IDL, LDL, and HDL from the diabetic rabbits.

Insulin treatment for a 10-day interval produced a 90% reduction in plasma triglycerides in the diabetic rabbits, yet cholesterol levels remained elevated at levels found in the non-diabetic control rabbits. Insulin treatment of the diabetic rabbits resulted in d < 1.006 g/ml plasma lipoproteins with compositions similar to those in non-diabetic, cholesterol-fed rabbits where β-VLDL is the most prominent cholesterol-carrying lipoprotein (14–16). The effect of insulin treatment of causing the accumulation of β-VLDL rather than triglyceride-rich lipoproteins in the diabetic rabbits may explain the earlier observations of Duff, Brechin, and Finkelstein (2), who showed that insulin treatment of diabetic rabbits reverses this animal's resistance to diet-induced atherosclerosis.

The present data are consistent with the conclusion that the alterations in plasma lipoproteins of the diabetic rabbit, produced in response to consumption of a diet enriched with cholesterol and fat, are due to the accumulation of partially metabolized, intestinally derived lipoproteins. That insulin treatment can correct these alterations suggests an important role for this hormone in the normal metabolism of chylomicrons. ∎

TABLE 6. Effect of insulin on composition of d < 1.006 g/ml lipoproteins in cholesterol-fed diabetic rabbits

Lipoproteins	Composition[a]	
	S_f 400	VLDL
Protein	3.0 ± 0.0	7.8 ± 3.8
Free cholesterol	7.5 ± 0.7	9.5 ± 0.9
Cholesteryl ester	54.5 ± 7.3	46.0 ± 7.1
Triglycerides	20.5 ± 9.0	20.5 ± 8.2
Phospholipids	10.8 ± 0.9	16.3 ± 0.3
Total cholesterol[b]	23.1 ± 9.5	18.8 ± 3.8
Triglycerides	23.5 ± 9.3	21.9 ± 3.6

[a]Percent of total mass; mean ± SD; n = 4 after insulin treatment.
[b]Percent of total plasma cholesterol or triglycerides recovered in fraction.

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