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, $S_I > 400$ plasma lipoproteins in the diabetic rabbits, and to $\beta$-VLDL in control rabbits. $S_I > 400$ lipoproteins, VLDL, IDL, LDL, and HDL from diabetic rabbits had triglyceride as the predominant lipoprotein core lipid. $S_I > 400$ 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. $S_I > 400$ lipoprotein and VLDL triglyceride content decreased but remained a major core lipid. Fasting eliminated apoproteins A-I and A-IV from $S_I > 400$ 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 $\beta$-VLDL both in the $S_I > 400$ lipoprotein and VLDL fractions. These results indicate that the alterations in plasma lipoproteins in cholesterol-fed diabetic rabbits result from the presence in the $d < 1.006 \text{ g/ml}$ 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

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 $d < 1.019 \text{ g/ml}$ plasma lipoproteins (4). We examined the chemical and physical properties of the $d < 1.019 \text{ g/ml}$ 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 $\beta$-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 for study. Two weeks after treatment, the diabetic rabbits' 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 K2EDTA 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% NaN3 and 0.01% K2EDTA (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. Sf > 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 Sf > 400 lipoproteins. VLDL, IDL, LDL, and HDL were isolated in sequence from the Sf > 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 Sf > 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 reisolated 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 apoprotein content of Sf > 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 Sf > 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% NaN3 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-ZnSO4 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% H2SO4 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 (x ± 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 Sf > 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 (Sf > 400 and VLDL) from diabetic, and 61% from control rabbits. The diabetic rabbits had significantly more Sf > 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/cholesterol esters in the Sf > 400 and VLDL fractions in the diabetic animals were 2.0 ± 0.3 and 1.7 ± 0.3 (x ± SD), respectively. Plasma cholesterol levels remained unchanged after 48 hr of fasting in the diabetic rabbits. There was a slight decrease in Sf > 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 Sf > 400 lipoprotein fraction, which lost 60% of its triglyceride mass. The molar ratio of triglyceride/cholesterol ester decreased upon fasting to less than 1.0 in both Sf > 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. Sf > 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 (Sf > 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 Sf > 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 Sf > 400 particles appeared heterogenous with the main fraction eluting at the void volume (Fig. 2A). VLDL was included in the
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 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, 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.

### 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

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Plasma</th>
<th>( S_f &gt; 400 )</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed diabetic</td>
<td>1837 ± 37</td>
<td>1029 ± 27</td>
<td>188 ± 37</td>
<td>20 ± 7</td>
<td>26 ± 8</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Fed control</td>
<td>1485 ± 372</td>
<td>518 ± 220</td>
<td>388 ± 110</td>
<td>230 ± 33</td>
<td>167 ± 45</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Fasted diabetic</td>
<td>1771 ± 98</td>
<td>1032 ± 75</td>
<td>351 ± 84</td>
<td>45 ± 16</td>
<td>66 ± 27</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Fasted control</td>
<td>1269 ± 365</td>
<td>305 ± 130</td>
<td>503 ± 177</td>
<td>148 ± 20</td>
<td>99 ± 21</td>
<td>16 ± 3</td>
</tr>
</tbody>
</table>

### Table 2: Percent lipid composition of lipoproteins from fed and fasted diabetic and control rabbits

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>C</td>
<td>6.8 ± 1.0</td>
</tr>
<tr>
<td>IDL</td>
<td>CE</td>
<td>4.0 ± 0.1</td>
</tr>
<tr>
<td>LDL</td>
<td>TG</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>HDL</td>
<td>PL</td>
<td>11.3 ± 1.9</td>
</tr>
</tbody>
</table>

*Values are mean ± SE; n = 4 in each group.

\( P < 0.05 \) compared to control rabbits (Mann-Whitney U-test).

\( P < 0.01 \) compared to corresponding fasted animal (paired t-tests).

\( P < 0.001 \) compared to corresponding fasted animal (paired t-tests).

C, unesterified cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid.

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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

<table>
<thead>
<tr>
<th></th>
<th>Fed Diabetic</th>
<th>Fasted Diabetic</th>
<th>Fed Control</th>
<th>Fasted Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sf &gt; 400</td>
<td>19.5 ± 1.9</td>
<td>10.6 ± 1.0*</td>
<td>4.6 ± 1.3</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>VLDL</td>
<td>19.4 ± 3.2</td>
<td>10.7 ± 1.7</td>
<td>5.5 ± 0.8</td>
<td>5.7 ± 1.4</td>
</tr>
</tbody>
</table>

*Values are the mean ± SE from four diabetic and three control rabbits.

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 Sf > 400 lipoproteins and VLDL from fed and fasted dia-

![Fig. 2. Elution patterns of Sf > 400 (A) and VLDL (B) lipoproteins from a cholesterol-fed diabetic rabbit on a column of 2% agarose. Lipoprotein cholesterol (A-A, Sf > 400; B-B, VLDL) and absorbance (Δ-Δ, Sf > 400; Δ-Δ, VLDL) were determined on individual fractions. Each plasma lipoprotein fraction was chromatographed separately. Vo indicates void volume of the column.](image-url)
abetic rabbits. ApoB-100 was present in both Sf > 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.

Apolipoprotein 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. Apolipoprotein A-I was the dominant apolipoprotein in HDL from both types of animals. Fasting did not affect the apolipoprotein profiles of IDL, LDL, and HDL in either diabetic or control rabbits, nor did it affect the apolipoprotein profile of Sf > 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 Sf > 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 Sf > 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 apolipoprotein 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

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Fig. 3. Elution pattern of Sf > 400 lipoprotein cholesterol (●●●●) and absorbance (○○○○) from a cholesterol-fed diabetic rabbit on a column of 1% agarose. Vo indicates void volume of the column.

Fig. 4. SDS-polyacrylamide gel (10%) electrophoretograms of Sf > 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 Sf > 400 and VLDL lipoproteins was obtained during ad lib feeding, and the right member after a 48-hr fast; alb, albumin.
cholesterol remained in the S₄ > 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 S₄ > 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 S₄ > 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 metabolism of chylomicrons and VLDL was defective in these animals.

Both apoB-100 and apoB-48 were present in S₄ > 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 S₄ > 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.](image1)

![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.](image2)
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 lipolysis, 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 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, lipolysis 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 5. Effect of insulin on plasma glucose, cholesterol, and triglyceride concentration in cholesterol-fed diabetic rabbits**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Glucose*</th>
<th>Cholesterol*</th>
<th>Triglycerides*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PZI</td>
<td>418 ± 21</td>
<td>1847 ± 272</td>
<td>8051 (2257–20128)</td>
</tr>
<tr>
<td>After PZI</td>
<td>74 ± 15</td>
<td>2016 ± 524</td>
<td>774 (242–6601)</td>
</tr>
</tbody>
</table>

*Mean ± SE, mg/dl, n = 5.

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

<table>
<thead>
<tr>
<th>Lipoproteins</th>
<th>Sf &gt; 400</th>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>3.0 ± 0.0</td>
<td>7.8 ± 3.8</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>7.5 ± 0.7</td>
<td>9.5 ± 0.9</td>
</tr>
<tr>
<td>Cholesteryl ester</td>
<td>54.5 ± 7.3</td>
<td>46.0 ± 7.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>20.5 ± 9.0</td>
<td>20.5 ± 8.2</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>10.8 ± 0.9</td>
<td>16.3 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>23.1 ± 9.5</td>
<td>18.8 ± 3.8</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>23.5 ± 9.3</td>
<td>21.9 ± 3.6</td>
</tr>
</tbody>
</table>

*Percent of total mass; mean ± SD; n = 4 after insulin treatment.

*Percent of total plasma cholesterol or triglycerides recovered in fraction.
REFERENCES


