# Serum Lipoproteins of Diabetic Rats Fed a High Cholesterol Diet

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**SUMMARY**

**Feeding a diet containing 2% cholesterol and 1% cholic acid (wt/wt) to rats made diabetic by administration of streptozotocin (45 mg/kg) produced marked hypercholesterolemia characterized by high concentrations of very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) and a reduction in concentration of high density lipoproteins (HDL). The VLDL was unique in that it contained apo A-I and apo A-IV in addition to its usual complement of apoproteins: apo B, apo E, and the C apoproteins. IDL had a similar apoprotein composition. The HDL from these rats was deficient in apo E. Nondiabetic rats fed the same diet exhibited similar qualitative changes in lipoprotein concentration and composition but with lesser increases in VLDL and IDL concentrations. The altered apoprotein composition suggested that the hyperlipoproteinemia associated with cholesterol feeding in the rat is due to an inadequate rate of removal of lipoproteins of intestinal origin, and that this is greatly exacerbated by diabetes. DIABETES 29:774–777, October 1980.**

We have previously reported that diabetic rats fed sucrose-rich diets develop marked hyperlipoproteinemia with alterations in the apoprotein composition of their lipoproteins.[1,2] By feeding diabetic rats a diet containing cholesterol and cholic acid, hyperlipoproteinemia is produced which differs markedly from that found with the sucrose-rich diet. With the cholesterol-cholic acid diet, rats develop severe hypercholesterolemia with marked alterations in the type and concentrations of the serum lipoproteins. In addition, the apoprotein composition of all lipoprotein classes was altered. The present studies were designed to compare the effects of diets containing cholesterol and cholic acid in diabetic and nondiabetic rats.

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## MATERIALS AND METHODS

**Animals and diet.** Sprague-Dawley male rats (Holtzman Laboratories, Madison, Wisconsin) weighing 175–250 g were caged individually and allowed free access to food and water throughout the study. Two diets were used: (1) a standard pelleted laboratory chow diet (Ralston Purina), and (2) a powdered diet (Teklad Mills, Madison, Wisconsin) containing (wt/wt) 50% cornstarch, 21% casein, 10% cellulose, 10% lard, 2% cholesterol, and 1% cholic acid, plus USP XIV mineral and Teklad #40060 vitamin mixtures (the cholesterol-cholic acid diet). After 1 wk on chow, rats were intravenously injected with streptozotocin (45 mg/kg body wt), as described by Bar-On et al.[1] A second group of rats was sham injected with citrate buffer, which did not contain streptozotocin. The diabetic and nondiabetic rats were fed the chow diet for an additional week and then placed on the cholesterol-cholic diet for 3 wk. An additional group of nondiabetic rats was maintained on chow for 3 wk. At the end of this period, the animals were anesthetized with ether and killed by exsanguination from the abdominal aorta without prior fasting.

**Lipoprotein separation and apoprotein characterization.** Serum was fractionated into the following density classes—very low density lipoproteins (VLDL), d < 1.006 g/ml; intermediate density lipoproteins (IDL), d 1.006–1.030 g/ml; low density lipoproteins (LDL), d 1.030–1.063 g/ml; and high density lipoproteins (HDL), d 1.063–1.210 g/ml—by the method of Havel et al.[3] and purified by recentrifugation at the higher density. The densities chosen for separation of the lipoproteins of the cholesterol-fed rats were those defined by Lasser et al.[4] The lipoprotein fractions were dialyzed and delipidated by solvent extraction with ethanol:ether (3:1 vol/vol), as described by Bar-On et al.[2] The apoproteins obtained were separated by sodium dodecyl sulfate (SDS) slab gel electrophoresis according to the

TABLE 1
Serum insulin, glucose, cholesterol, and triglyceride concentrations of diabetic and nondiabetic rats

| | Insulin (μU/ml) | Glucose (mg/dl) | Cholesterol (mg/dl) | Triglyceride (mg/dl) |
|---|---|---|---|---|
| Nondiabetic rats | | | | |
| Chow (N = 3) | 71.3 ± 32.3 | 157 ± 4.9 | 49 ± 2.3 | 85.3 ± 15.0 |
| Cholesterol-cholic acid diet (N = 6) | 56.6 ± 20.8 | 177 ± 18.3 | 424 ± 38.1 | 134.5 ± 55.7 |
| Diabetic rats | | | | |
| Cholesterol-cholic acid diet (N = 5) | 23.3 ± 1.3 | 497 ± 27.4 | 1376 ± 395.6 | 474.8 ± 211.0 |

Each value represents the mean ± SD.

method of Swaney and Kuehl,[5] utilizing gels with a gradient of 3–27% acrylamide. Isoelectric focusing was performed by the method of Gidez et al.[6]

**Chemical analyses.** Serum glucose was determined using a Beckman glucose analyzer. Serum insulin levels were assayed according to the method of Herbert et al.[7] Lipids, for the determination of triglyceride and phospholipid in serum and lipoprotein fractions, were extracted according to the method of Folch et al.[8] Triglycerides were measured by the procedure of Van Handel and Zilversmit as modified by Van Handel.[9] Phospholipids were determined by the method of Zilversmit and Davis.[10] Serum and lipoprotein cholesterol were measured by the method of Abell et al.[11] Free and ester cholesterol were measured by the method of Sperry and Webb,[12] and protein content of lipoprotein fractions was determined by the method of Lowry et al.[13] as modified by Sata et al.[14]

## RESULTS

The presence of diabetes in the streptozotocin-treated rats was confirmed by the finding of elevated serum glucose concentrations and reduced concentrations of serum insulin (Table 1). Serum cholesterol and triglyceride concentrations were elevated in the nondiabetic rats fed the cholesterol-cholic acid diet, but were considerably higher in the diabetic rats fed the same diet (Table 1).

Measurement of protein and cholesterol distribution in the lipoprotein fractions revealed marked increases in the lower density classes after cholesterol feeding. VLDL protein concentration was increased in the rats fed the cholesterol-cholic acid diet, and was increased considerably more in the diabetic rats (Table 2). IDL protein, which was not detected in the chow-fed nondiabetic rats, was present in appreciable amounts in the nondiabetic rats fed the cholesterol-cholic acid diet, and was fivefold higher in the diabetic rats. LDL protein was increased in the nondiabetic rats fed the cholesterol-cholic acid diet and much more so in the diabetic rats on this diet. HDL protein was reduced by 60% in both groups. VLDL and IDL cholesterol concentrations increased in rats fed the cholesterol-cholic acid diet, but were considerably higher in the diabetic rats. LDL cholesterol was increased in the nondiabetic rats fed cholesterol-cholic acid and very markedly increased in the diabetic rats fed this diet. HDL cholesterol concentration decreased in both groups fed the cholesterol-cholic acid diet. The ratio of cholesterol to protein of all lipoprotein fractions of rats fed the cholesterol-cholic acid diet was higher than in lipoproteins of chow-fed rats. The pool obtained from the cholesterol-fed rats showed no increase in VLDL triglyceride. However, in the cholesterol-fed diabetic rats the VLDL triglyceride increased markedly, and triglyceride was also higher in IDL.

Table 3 shows a complete characterization of the constituents of the lipoproteins. The proportion of core lipid (triglyceride and cholesteryl esters) in VLDL was similar in all

TABLE 2
Protein, cholesterol, and triglyceride in serum lipoproteins of chow-fed, cholesterol-fed, and cholesterol-fed diabetic rats*

| | VLDL | IDL | LDL | HDL | Total |
|---|---|---|---|---|---|
| | (mg/dl serum) | | | | |
| Protein | | | | | |
| Chow-fed | 6.3 | — | 4.6 | 61.7 | 72.6 |
| Cholesterol-fed | 25.6 | 21.0 | 7.2 | 22.8 | 76.6 |
| Cholesterol-fed diabetic | 114.1 | 110.0 | 30.6 | 26.5 | 281.2 |
| Cholesterol | | | | | |
| Chow-fed | 1.6 | — | 2.1 | 29.2 | 33.0 |
| Cholesterol-fed | 213.7 | 77.8 | 11.6 | 13.9 | 317.0 |
| Cholesterol-fed diabetic | 874.1 | 439.2 | 64.0 | 17.0 | 1394.0 |
| Triglyceride | | | | | |
| Chow-fed | 56.0 | — | 1.6 | — | 57.6 |
| Cholesterol-fed | 55.0 | 5.0 | 2.0 | — | 62.0 |
| Cholesterol-fed diabetic | 474.0 | 19.0 | 2.0 | — | 495.0 |

* Pooled samples of serum from three to six rats were analyzed.

TABLE 3
Chemical composition of lipoproteins in chow-fed, cholesterol-fed (CF), and cholesterol-fed diabetic (CFD) rats*

| | VLDL | IDL | LDL | HDL |
|---|---|---|---|---|
| | (% by wt) | | | |
| Chow | | | | |
| UC† | 1.1 | —‡ | 4.8 | 2.4 |
| CE | 3.1 | — | 25.7 | 33.8 |
| PL | 10.1 | — | 20.0 | 19.8 |
| TG | 78.8 | — | 15.1 | — |
| Protein | 6.9 | — | 34.2 | 44.0 |
| CF | | | | |
| UC† | 3.0 | 6.8 | 4.5 | 1.4 |
| CE | 46.6 | 50.6 | 39.8 | 25.1 |
| PL | 9.1 | 16.4 | 18.5 | 12.1 |
| TG | 32.3 | 4.6 | 7.4 | — |
| Protein | 9.0 | 21.6 | 29.8 | 60.8 |
| CFD | | | | |
| UC† | 6.1 | 8.4 | 8.9 | 4.4 |
| CE | 55.2 | 73.6 | 48.3 | 29.4 |
| PL | 12.5 | 2.8 | 20.0 | 22.1 |
| TG | 22.0 | 2.1 | 1.1 | — |
| Protein | 4.2 | 13.1 | 21.7 | 44.1 |

* Pooled samples of serum for eight rats were analyzed.
† Abbreviations: UC, unesterified cholesterol; CE, cholesteryl esters; PL, phospholipid; and TG, triglyceride.
‡ IDL (d 1.006–1.03 g/ml) is not present in chow-fed rats.

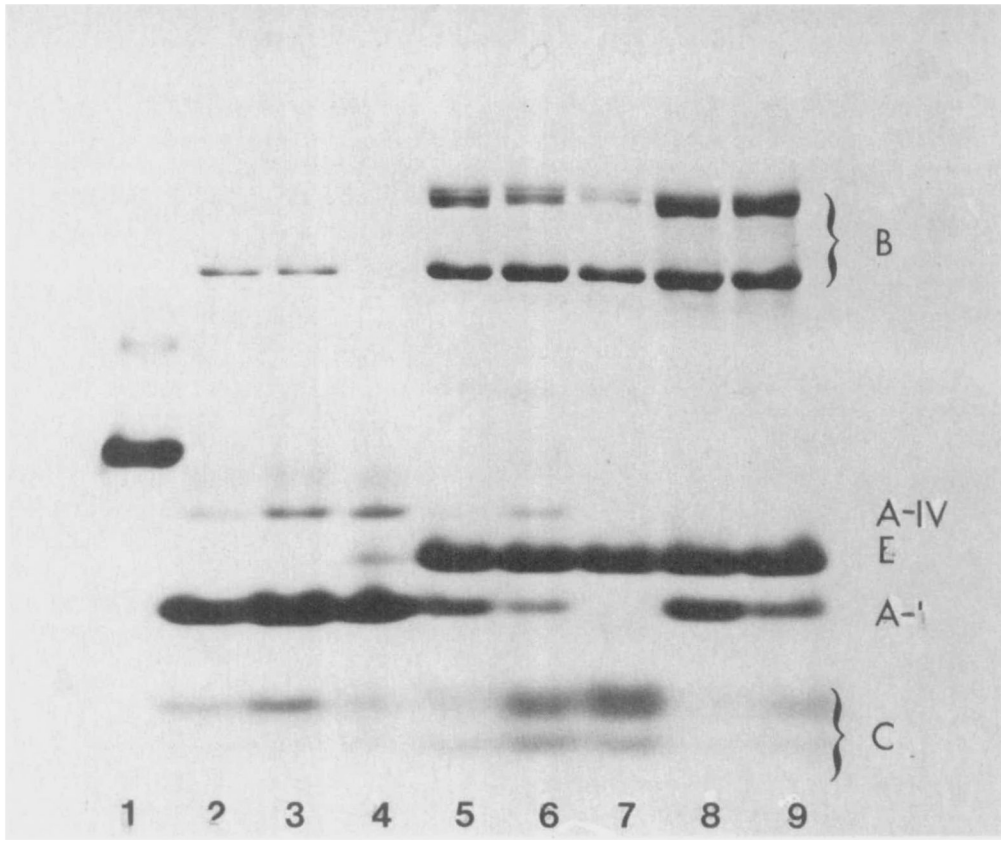


**FIGURE 1. SDS gel electrophoresis of delipidated lipoproteins. An aliquot containing 75 μg of lipoprotein-protein was applied to each position. Position 1 contained bovine serum albumin. Position 2, HDL from diabetic rats fed cholesterol-cholic acid; position 3, HDL from nondiabetic rats fed cholesterol-cholic acid; position 4, HDL from chow-fed rats; position 5, VLDL from diabetic rats fed cholesterol-cholic acid; position 6, VLDL from nondiabetic rats fed cholesterol-cholic acid; position 7, VLDL from chow-fed rats; position 8, IDL from nondiabetic rats fed cholesterol-cholic acid; and position 9, IDL from nondiabetic rats fed cholesterol-cholic acid.**

three groups. However, in the cholesterol-fed rats the proportion of cholesteryl ester increased and was more marked in the cholesterol-fed diabetic rats. IDL from cholesterol-fed rats contained more than 50% core lipid while IDL from cholesterol-fed diabetic rats contained 75% core lipids, an amount similar to that in VLDL from all groups. The LDL of both cholesterol-fed rats contained less triglyceride than in the chow-fed rats. HDL was quite similar in overall composition in all three groups.

SDS gel electrophoresis (Figure 1) of VLDL from diabetic (position 5) and nondiabetic (position 6) rats fed the cholesterol-cholic acid diet contained bands with electrophoretic mobilities corresponding to apo A-I and apo A-IV. These bands were not present in the VLDL from serum of chow-fed rats (position 7). Apo E, apo B, and apo C were present in VLDL from all three groups. IDL from the diabetic (position 8) and nondiabetic rats (position 9) had an apoprotein pattern similar to that of VLDL. HDL of diabetic (position 2) and nondiabetic (position 3) rats fed the cholesterol-cholic acid diet was deficient in apo E, a component normally present in rat HDL (position 4). LDL prepared from the diabetic and nondiabetic rats fed the cholesterol-cholic acid diet contained appreciable amounts of protein corresponding to apo A-I and apo E. The identification of apo A-I in the VLDL and IDL was verified by isoelectric focusing, which showed the same two major isoforms present in apo A-I of HDL from chow-fed rats.

## DISCUSSION

This study indicates that induction of diabetes with streptozotocin results in a marked accentuation of the hypercholesterolemia produced by feeding with a diet containing cholesterol and cholic acid. Concentrations of VLDL were increased in the nondiabetic rats and an IDL fraction (d 1.006–1.030 g/ml) appeared. In the diabetic rats, both VLDL and IDL concentrations increased to a much greater extent. In previous studies from this laboratory, using diets containing 1% cholesterol and 10% olive oil, IDL was found, although much lower levels of cholesterol were attained.[4] It would thus appear that the presence of IDL is a usual consequence of cholesterol feeding in the rat. The VLDL obtained from both diabetic and nondiabetic cholesterol-fed rats does not differ appreciably in composition. The proportion of core lipid in each is very similar. However, IDL in the cholesterol-fed diabetic rat has a much higher proportion of core lipid than the IDL of the cholesterol-fed rats, suggesting that the size of the particles differs in the two types of cholesterol-fed rats. This will require confirmation by agarose chromatography and also by direct electron microscopy. The reasons for this difference in IDL are not apparent. The VLDL and IDL of the diabetic and nondiabetic rats fed the cholesterol-cholic acid diet contained apo A-I and apo A-IV, apoproteins not normally present in VLDL. Kuehl et al.[15] observed similar changes in rats fed cholesterol and olive oil.

It is of interest to speculate on the origin of the IDL. One

likely possibility is that it is a remnant derived from the chylomicrons. However, Riley et al.[16] have recently found IDL containing apo A-I in the intestinal lymph of cholesterol-fed rats, suggesting that the IDL found in the plasma may be derived directly from the intestine.

The HDL of the hypercholesterolemic rats was decreased in concentration and deficient in apo E, a finding noted by Mahley and Holcombe.[17] These investigators have also isolated a fraction with $\alpha_2$ mobility, which contained apo A-I and apo E in the d 1.030–1.063 g/ml fraction, and have designated this fraction $HDL_c$. The finding of apo A-I and apo E at this density range in our studies is probably due to the presence of $HDL_c$ in this fraction, since these apoproteins are not found at this density range in chow-fed rats.

The effects of feeding a cholesterol-cholic acid diet to diabetic rats were quite different from those observed with feeding a high sucrose diet.[1,2] As shown by the protein determinations, the concentrations of the various low density lipoprotein classes were considerably higher in the diabetic rats fed the cholesterol-cholic acid diet. VIDL protein was 60% higher in the cholesterol-cholic acid group. No IDL was found in the sucrose-fed diabetic rats, whereas this fraction was markedly elevated in rats fed the cholesterol-cholic acid diet. A possible explanation for this difference is that the VLDL of sucrose-fed rats has a very high triglyceride content, and lipolysis produces a particle with relatively low lipid content, which is rapidly removed from the circulation. However, in the cholesterol-cholic acid–fed rats, much of the triglyceride is replaced by cholesteryl ester;[4] this lipid is not removed and remains as the IDL fraction after the metabolism of VLDL. Therefore, the very high concentration of VLDL following cholesterol feeding may result in a saturation of removal mechanisms for remnants, which Sherrill and Dietchy[18] have shown in isolated liver perfusion studies to be saturated at cholesterol levels of 12 mg/dl of perfusate.

The apoprotein composition of VLDL in diabetic rats fed the cholesterol-cholic acid diet differed from that of the diabetic rats fed the sucrose-enriched diet. Rats fed a sucrose-rich diet did not have apo A-I in VLDL, in contrast to diabetic and nondiabetic rats fed the cholesterol-cholic acid diet. Furthermore, in the sucrose-fed diabetic rats, we observed alterations in the distribution of C-III apoproteins of VLDL, with a shift to more highly sialylated forms. This was not seen in rats fed the cholesterol-cholic acid diet.

In contrast to sucrose feeding, HDL concentrations of diabetic and nondiabetic rats fed the cholesterol-cholic acid diet were decreased to the same extent, whereas, in rats fed the sucrose-rich diet, induction of diabetes resulted in an increase in HDL protein. Furthermore, the HDL of diabetic rats fed the sucrose-rich diet was deficient in apo A-IV, but in diabetic rats fed the cholesterol-cholic acid diet this apoprotein was present. The HDL of diabetic sucrose-fed rats was deficient in apo E, which was also noted in both diabetic and nondiabetic rats fed the cholesterol-cholic acid diet.

The finding of significant amounts of apo A-I and apo A-IV in the VLDL and IDL of the cholesterol-fed rats suggests that these particles may be of intestinal origin, since apo A-I and apo A-IV are synthesized by the intestine and appear in intestinal chylomicrons, VLDL, and IDL in cholesterol-fed rats.[16,19,20] Ordinarily the apo A-I and apo A-IV leave the chylomicrons when they enter the circulation. These particles may accumulate in the serum of cholesterol-fed rats because of an inability to remove them at a sufficiently rapid rate. The mechanism whereby diabetes accentuates this process is presently being investigated.

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