Lipoprotein Levels in Genetically Selected Mice with Increased Susceptibility to Atherosclerosis

W. Carl Breckenridge, Alexander Roberts, and Arnis Kuksis

Male mice of two inbred strains, C57BR/cdJ and CBA/J, were maintained either on an atherogenic diet rich in cholesterol (5%) and saturated fat (30%) or on a control Purina Laboratory Chow diet. After 3 weeks on a 90% atherogenic/10% chow diet, the C57BR/cdJ mouse showed a fivefold increase in plasma total cholesterol level, while the CBA/J strain indicated only a twofold increase. On the atherogenic diet, both groups of animals showed marked increases in a lipoprotein of a broad density spectrum (1.006 > d < 1.063) with prebeta and beta mobility on agarose gel electrophoresis, but the C57BR/cdJ strain showed a greater increase than the CBA/J strain. The very low density lipoprotein (VLDL) contained predominantly lipoprotein of prebeta mobility, while intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) contained lipoprotein of beta-mobility. In both strains, the major lipid component in this lipoprotein was cholesteryl ester and both strains showed large amounts of an apoprotein (apo) with E mobility as defined for apo B proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis or by isoelectric focusing on polyacrylamide-urea disc gels. The phosphatidylcholine/free cholesterol ratio of the VLDL, IDL, and LDL fractions isolated from the C57BR/cdJ mice on the atherogenic diet (0.76 to 0.95) was noticeably lower than that (1.10 to 1.19) from the CBA/J mice maintained on the same diet. There was a marked decrease in the high density lipoprotein (HDL) fraction in both strains of mice on the atherogenic diet, but a greater reduction in the HDL level of the C57BR/cdJ strain with a 50% decrease in the phosphatidylcholine/free cholesterol ratio. It is suggested that the development of atherosclerosis in the C57BR/cdJ mice on the atherogenic diet results from a greatly increased accumulation of the cholesteryl ester-rich VLDL and IDL and a depletion of HDL, all of which are characterized by decreased phosphatidylcholine/free cholesterol ratios when compared to those of the CBA mice. (Arteriosclerosis 5:256–264, May/June 1985)

Extensive studies of plasma lipoproteins have shown that lowered low density lipoproteins (LDL) and elevated very low density lipoproteins (VLDL) are positively correlated with increased risk for ischemic vascular and coronary heart disease in humans and that high density lipoproteins (HDL) are associated with reduced risk and possible protection against atherosclerotic disease. In addition, it has been shown that a low plasma phosphatidylcholine/free cholesterol ratio has higher correlation with ischemic vascular disease in humans than does HDL cholesterol. We have recently reported that in an inbred strain of mice on a hypercholesterolemic diet a low plasma phosphatidylcholine/free cholesterol ratio precedes or accompanies the development of atheromas. It has not been established whether the abnormal phosphatidylcholine/free cholesterol ratio is associated with the accumulation of any specific plasma lipoproteins or is characteristic of all lipoproteins under the disease conditions.

The present work shows that the development of atheromas in the susceptible strain of the inbred mice is associated with an accumulation of VLDL and intermediate density lipoproteins (IDL), and with a depletion of HDL and that all lipoproteins have phosphatidylcholine/free cholesterol ratios that are significantly lower in the susceptible than in the resistant mice.
LIPOPROTEIN LEVELS IN ATHEROSCLEROSIS-SUSCEPTIBLE MICE

Breckenridge et al.

Methods

Animals

Male mice of the C57BR/cdJ (susceptible) and CBA/J (resistant) strains were purchased from the Jackson Laboratories, Bar Harbor, Maine, when they were 6 weeks old and were placed on Purina Laboratory Chow (containing 4.5% crude fat) and water ad libitum. At 10 weeks of age, mice of both strains were divided into treatment and control groups. The treatment groups were started on a diet consisting of 70% atherogenic (high fat, high cholesterol) food and 30% Purina Laboratory Chow for 2 days, then a 80% atherogenic and 20% chow diet for 4 days, followed by a 90% atherogenic and 10% chow diet for various periods of time until sacrificed. The animals accepted the diet readily. The corresponding control groups were maintained for the same periods of time on Purina Laboratory Chow.

Lipoprotein Isolation

The animals were fasted overnight and sacrificed by using intraperitoneal sodium pentobarbital anesthesia (0.02 ml/g body weight). Whole blood (0.5 to 1.0 ml per animal) was removed from the inferior vena cava, was mixed with solid ethylenediaminetetraacetic acid (EDTA, 1.2 mg/ml blood) and was immediately chilled on ice. For the preparation of lipoproteins, blood samples were pooled separately from 16 C57BR/cdJ mice on the 90% atherogenic, 10% chow diet, from 19 C57BR/cdJ mice on the chow diet, and from 20 CBA/J mice on each of the atherogenic and the chow diets. Lipoprotein fractions were obtained by ultracentrifugation at selected densities in a Beckman rotor (40:3) as follows: VLDL (d < 1.006) at saline density for 18 hours at 40,000 rpm; IDL at 1.006 < d < 1.019 for 20 hours at 40,000 rpm; LDL at 1.019 < d < 1.063 for 20 hours at 40,000 rpm, and HDL at 1.063 < d < 1.21 for 48 hours at 40,000 rpm. Lipoproteins were separated by agarose gel electrophoresis as described elsewhere. Isolated lipoproteins were also characterized by gel filtration on Beckman TSK columns as described previously. Apoprotein patterns were determined by isoelectric focusing after delipidation with ethanol-diethyl ether and solubilization of the apoproteins in the urea. Alternatively, the apoproteins were solubilized in 1% sodium dodecyl sulfate containing dithioerythritol and were resolved with sodium dodecyl sulfate polyacrylamide gel electrophoresis by using 4% to 20% gradient polyacrylamide gels and 0.2 M Tris glycine buffer (pH 8.3) containing 0.1% sodium dodecyl sulfate.

Total Lipid Profile Determination

The total lipid profiles of the various lipoprotein fractions were determined as previously described. For this purpose, a neutral lipid extract was prepared as follows. An aliquot of the fraction equivalent to about 0.2 ml (0.5 ml of the solution) of plasma was added to a Teflon-lined, screw-cap centrifuge tube containing 0.2 to 0.4 ml phospholipase C (C. welchii, Sigma Chemical Company, St. Louis, Missouri) in 4 ml of 17.5 mM Tris buffer (pH 7.3) along with 1.3 ml of 1% CaCl2 and 1 ml of diethyl ether. The mixture was incubated with stirring for 2 hours at 30° C. The incubation mixture was then treated with 5 drops of 0.1 N HCl and extracted once by vigorous shaking with 10 ml of chloroform/methanol (2:1) containing 100 μg tridecanoylglycerol as the internal standard. The final dried extract was dissolved and silylated in 150 μl to 250 μl of TRI-SIL/BSA (Pierce Chemical Company, Rockford, Illinois), and an aliquot was injected into the gas chromatograph.

Other Methods

The serum total cholesterol level was determined by the Technicon Auto-Analyzer Method N-24a as previously described. The protein content of the lipoprotein fractions was determined by the method of Lowry et al., with diethyl-ether extraction following color development or by a modification that included sodium dodecyl sulfate in the reagents. Bovine serum albumin was used as a standard.

Results

Total Cholesterol Analyses

Table 1 summarizes the mean serum total cholesterol levels of the susceptible and resistant strains of mice maintained on the atherogenic and the control diets for 3, 5, 10, and 15 weeks. On the experimental diets, both the susceptible and the resistant mice had significantly higher cholesterol levels than on the control diet. Furthermore, the susceptible mice had significantly higher cholesterol levels than the resistant mice on both the atherogenic and the chow diet at all times. These values are similar to those estimated earlier from the plasma total lipid profiles of these inbred strains of mice.

Table 2 gives the distribution of total cholesterol in the lipoproteins isolated from plasma pooled from 16 to 20 susceptible and resistant mice. For both strains on the chow diet, over 90% of the plasma total cholesterol was in the HDL fraction, with extremely low values in the VLDL and LDL fractions. There was not enough IDL to be measured. After 3 weeks on the 90% atherogenic, 10% chow diet, the susceptible mice had most of the total plasma cholesterol in the VLDL, IDL, and LDL fractions, with the highest proportion in IDL. The resistant mice on the atherogenic diet also showed a marked increase in VLDL, IDL, and LDL cholesterol levels, but the highest proportion occurred in the VLDL fraction. Both strains of mice showed a significant decrease in the HDL fraction, with the susceptible strain undergoing the greatest change. HDL cholesterol in the resistant mice was reduced by one-half and that in the susceptible mice by five-sixths.
Table 1. Plasma Cholesterol Levels in Mice Given Atherogenic and Chow Diets

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Diet</th>
<th>3 weeks</th>
<th>5 weeks</th>
<th>10 weeks</th>
<th>15 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BR/cdJ (susceptible)</td>
<td>Atherogenic</td>
<td>412.2 ± 41.1</td>
<td>444.5 ± 52.1</td>
<td>286.2 ± 13.1</td>
<td>428.0 ± 33.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>90.1 ± 3.7</td>
<td>115.8 ± 9.3</td>
<td>109.2 ± 4.4</td>
<td>106.9 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(60–130)</td>
<td>(60–266)</td>
<td>(75–150)</td>
<td>(85–132)</td>
</tr>
<tr>
<td>CBA/J (resistant)</td>
<td>Atherogenic</td>
<td>170.0 ± 8.7</td>
<td>180.5 ± 7.3</td>
<td>212.2 ± 10.5</td>
<td>154.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>79.2 ± 3.3</td>
<td>90.2 ± 2.8</td>
<td>84.6 ± 4.2</td>
<td>91.2 ± 4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50–115)</td>
<td>(65–125)</td>
<td>(66–135)</td>
<td>(65–130)</td>
</tr>
</tbody>
</table>

Mean serum total cholesterol levels are given in mg/dl for the C57BR/cdJ and CBA/J strains after 3, 5, 10, and 15 weeks on the 90% atherogenic/10% chow diet and for the corresponding controls continued on the chow diet. The range of cholesterol values is given on the second line, and the size of the sample is given in parentheses. All samples are from individual mice.

Lipid Composition of Lipoprotein Classes

Figure 1 shows the gas-liquid chromatography (GLC) profiles of the HDL fractions of resistant and susceptible mice on the atherogenic and the chow diets. The corresponding quantitative results are recorded in Table 3. On the chow diet, the HDL fractions showed similar lipid molecular composition for both animal strains. Cholesteryl esters accounted for 45% to 47% of the lipid mass, phospholipids, for 47%, and cholesterol, for 3% to 5%. The lipid/protein ratio was slightly higher in the C57Br/cdJ than in the CBA/J strain. The relative ratios for the free and esterified cholesterol and for phosphatidyl choline and free cholesterol were similar to those found in human lipoproteins. Likewise, there was a similarity in the relative proportions of the carbon numbers of the diacylglycerols arising from phosphatidylcholine, and of the molecular species of the cholesteryl esters. There was readily detectable lysophosphatidylcholine in the HDL fractions from mice on both diets. In resistant mice on the atherogenic diet, the lipid class ratios in the HDL fraction were normal, while in the susceptible mice the phosphatidylcholine/free cholesterol ratio was 50% lower. There were slight increases in the proportion of cholesteryl ester and decreases in phospholipids, which were associated with an increase in the relative amount of large molecular weight HDL (Figure 2). However the HDL that accumulated during the atherogenic diet was still smaller than the LDL as assessed by gel filtration chromatography.

The susceptible strain showed a significantly lower proportion of cholesteryl esters containing fatty acids with 20 carbon atoms, compared to the HDL of the resistant strain. In contrast to the chow diet, the atherogenic diet brought about a significant increase in the C38 molecular species of diacylglycerol arising from phosphatidylcholines.

Figure 1. Gas-liquid chromatography (GLC) profiles of HDL lipids from resistant and susceptible strains of mice on atherogenic and control diets. A. Resistant (CBA/J) mouse on control diet. B. Susceptible (C57BR/cdJ) mouse on control diet. C. Resistant mouse on atherogenic diet. D. Susceptible mouse on atherogenic diet. Conditions of high temperature GLC are given in Reference 5. Peaks: 16–20, trimethylsilyl esters of free fatty acids, with 16–20 acyl carbons; 27, trimethylsilyl ether of cholesterol; 30, tridecanoylglycerol internal standard; 34, trimethylsilyl ether of palmitoylsphingosine; 36–40, trimethylsilyl ethers of diacylglycerols with a total number of 34–38 acyl carbons; 43–47, cholesteryl esters of fatty acids with a total number of 16–20 acyl carbons; 50–56, triacylglycerols with a total number of 50–56 acyl carbons. Sample size: 1 µl of approximately 1% solution in silylation mixture. Attenuation: 100 times full sensitivity.
Table 2. Distribution of Cholesterol in Mouse Lipoproteins from Pooled Samples

<table>
<thead>
<tr>
<th>Density fraction*</th>
<th>Control diet (mg%)†</th>
<th>Atherogenic diet (mg%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBA/J</td>
<td>C57BR/cdJ</td>
</tr>
<tr>
<td>Total plasma</td>
<td>105</td>
<td>96</td>
</tr>
<tr>
<td>d &lt; 1.006 (VLDL)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1.006 &gt; d &lt; 1.019 (IDL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.019 &gt; d &lt; 1.063 (LDL)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>1.063 &gt; d &lt; 1.21 (HDL)</td>
<td>101</td>
<td>89</td>
</tr>
</tbody>
</table>

*Plasma was pooled from 16 to 20 mice for each lipoprotein fraction. Numbers represent the density between which each fraction was isolated.
†Purina Laboratory Chow diet.
‡After 3 weeks on the 90% high-fat, high-cholesterol/10% chow diet.

Table 3. Composition of the Plasma Lipoproteins of the C57BR/cdJ and CBA/J Strains of Mice on the Atherogenic and Control Diets and Their Molar Phosphatidylcholine/Free Cholesterol Ratios

<table>
<thead>
<tr>
<th>Density fraction</th>
<th>Mouse strain</th>
<th>Lipoprotein constituents</th>
<th>Lipid/protein PC/FC (weight/weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FC</td>
<td>CE</td>
</tr>
<tr>
<td>Atherogenic diet</td>
<td>VLDL</td>
<td>d &lt; 1.006</td>
<td>C57BR/cdJ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.7</td>
<td>±3.5</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>d1.006–1.019</td>
<td>C57BR/cdJ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.1</td>
<td>±3.1</td>
</tr>
<tr>
<td></td>
<td>LDL</td>
<td>d1.019–1.063</td>
<td>C57BR/cdJ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.2</td>
<td>±3.4</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>C57BR/cdJ</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.3</td>
<td>±1.5</td>
</tr>
<tr>
<td></td>
<td>Control diet</td>
<td>VLDL</td>
<td>C57BR/cdJ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±1.1</td>
<td>±0.5</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>C57BR/cdJ</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBA/J</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.6</td>
<td>±1.5</td>
</tr>
</tbody>
</table>

Animals were tested after 3-week dietary periods, as in Table 1. The values are averages of two analyses of a single preparation of lipoproteins from pooled samples of plasma, as described under Methods. Lipoproteins were separated as described under Methods. Lipid classes were quantitated by high temperature GLC, as described previously (see reference 5). Protein was determined by the method of Lowry et al. (see reference 12). FC = free cholesterol; CE = cholesteryl esters; TG = triacylglycerols; PL = choline-containing phospholipids.
Figure 2. Resolution of lipoproteins by gel filtration chromatography. C57BR/cdJ mice on the atherogenic diet: VLDL (A), LDL (B), and HDL (C). C57BR/cdJ mice on the control diet: HDL (D). The separations were performed on Beckman TSK columns as previously described (see reference 8).

Figure 3 gives the total lipid profiles of the VLDL, IDL, and LDL fractions of the resistant and susceptible mice on the atherogenic diet. The lipoproteins showed certain similarities among the corresponding classes, but there were also marked differences. Thus, all mice showed only a trace of cholesteryl esters with C20 fatty acids. The molecular species of phosphatidylcholines containing C20 fatty acids were very low in the lipoproteins from animals fed the atherogenic diet. Quantitative data for these fractions are shown in Table 2 along with the VLDL from animals fed the control diet. The VLDL from the C57BR/cdJ on the control diet consistently had higher proportions of free and esterified cholesterol and a lower proportion of triacylglycerol compared with the CBA/J strain. For animals of both strains on the atherogenic diet, the VLDL was extremely rich in cholesteryl ester. The lipid/protein ratio for VLDL was lower from animals on the atherogenic diet than from those on the control diet. In contrast to HDL, the IDL and LDL from both strains of mice on the atherogenic diet had a decreased phosphatidyl choline/free cholesterol ratio, with a greater decrease in the susceptible strain (Table 3). The lipid class compositions of the IDL and LDL from both strains of animals on the atherogenic diet were similar; triacylglycerols, however, were higher in the CBA/J than in the C57BR/cdJ strain.

Figure 3. Gas-liquid chromatography (GLC) profiles of VLDL, IDL, and LDL lipids from resistant and susceptible strains of mice on atherogenic diet. A. VLDL of resistant (CBA/J) mice. B. IDL of resistant mice. C. LDL of resistant mice. D. VLDL of susceptible (C57BR/cdJ) mice. E. IDL of susceptible mice. F. LDL of susceptible mice. High temperature GLC conditions and peak identification are the same as for Figure 1.
C57BR/cdJ strain. It is possible that the IDL and LDL represent a broad spectrum of particles that differ primarily in the higher lipid/protein ratio in the IDL. In animals on the atherogenic diet, VLDL was greater than LDL, based on molecular weight as assessed by gel filtration chromatography (Figure 2).

**Electrophoretic Patterns of Lipoproteins and Apoproteins**

Figure 4 shows the results of agarose gel electrophoresis of the total plasma lipoproteins in the two strains of mice after 3 weeks on the 90% atherogenic, 10% chow diet or the control diet. On the chow diet, animals of both strains had only HDL with α-migration and very small amounts of a prebeta lipoprotein, as defined by electrophoretic mobility for human lipoproteins. After consumption of the atherogenic diet, the C57BR/cdJ (susceptible) strain showed a marked increase in the prebeta lipoproteins in the VLDL, IDL, and LDL fractions. With increasing density, there was some decrease in electrophoretic mobility of the lipoproteins tending toward beta-mobility. In the CBA/J (resistant) mice, there was a similar lipoprotein in smaller quantities in the VLDL, but the IDL and LDL fractions of those mice had a slower electrophoretic mobility, similar to β-mobility.

Apolipoproteins of VLDL and HDL were resolved on SDS-containing gradient gels (4% to 30%). Figure 5 shows that the HDL consisted primarily of one major apoprotein with a molecular weight (~30,000) similar to that of human apolipoprotein (apo) A-I. While there was a considerable amount of low molecular weight protein, apo E was not a major component. An unidentified protein with a molecular weight of approximately 50,000 was also present. Figure 6 shows that the mouse VLDL contained apo B, a protein with a molecular weight of approximately 35,000 to 40,000 corresponding to human apo E and other apolipoproteins of low molecular weight. Figures 7 and 8 show the results of isoelectric focusing of the apolipoproteins from the VLDL and HDL classes of the two strains of mice after consumption of the atherogenic diet. In qualitative agreement with the results of the SDS gel electrophoresis, the mouse VLDL contained relatively more apo E and apo C. The HDL contained a major apolipoprotein tentatively identified as apo A-I, which overlapped with apo E. This apolipoprotein had a lower molecular weight than the apo E characteristic of VLDL. This apolipoprotein had distinct isomorphic patterns in the mice of both strains. No attempt was made to characterize these apoproteins. It appears that the isoelectric point of mouse apo A-I is quite different from that of human apo A-I. The LDL contained only apo B, while the IDL had both apo E and apo B (data not given).

**Figure 4.** Agarose gel electrophoresis of plasma lipoproteins from resistant and susceptible mice on atherogenic and control diets. A. Susceptible (C57BR/cdJ) mice on control diet. B. Resistant (CBA/J) mice on control diet. C. Susceptible mice on atherogenic diet. D. Resistant mice on atherogenic diet. 1. plasma; 2. VLDL (d < 1.006); 3. IDL (1.006–1.019); 4. LDL (1.019–1.063); 5. HDL (1.063–1.12). The electrophoresis on the agarose gels was performed as described in Reference 7. a, b, and c refer to lipoproteins of α, prebeta, and β mobility, respectively. Note the increased prebeta lipoproteins in the VLDL, IDL, and LDL fractions of the susceptible mice on the atherogenic diet.

**Figure 5.** SDS polyacrylamide gradient gel (4% to 30%) electrophoresis of apolipoproteins from mouse HDL. A. Human HDL. B. HDL from CBA/J mice on the control diet. C. HDL from CBA/J mice on the atherogenic diet. D. HDL from C57BR/cdJ mice on the control diet. E. HDL from C57BR/cdJ mice on the atherogenic diet. Molecular weight markers at the left of the gel are: 1. lactalbumin (14,200); 2. soybean trypsin inhibitor (21,500); 3. carbonic anhydrase (31,000); 4. ovalbumin (45,000); 5. bovine serum albumin (66,200); 6. phosphorylase (92,500); 7. galactosidase (116,250); 8. myosin (200,000).
Figure 6. SDS-polyacrylamide gradient gel (4% to 30%) electrophoresis of apolipoproteins from mouse VLDL. Conditions are the same as in Figure 5. A. Human VLDL. B. CBA/J mice on the atherogenic diet. C. C57BR/cdJ mice on the control diet. D. C57BR/cdJ mice on the atherogenic diet.

Discussion

Many animal models have been developed for use in studying the induction and progression of atherosclerosis.14-17 The experimenters usually have fed the animals cholesterol and other dietary additives such as bile acids and propiothiouracil.14 Certain inbred strains of mice, however, readily develop hypercholesterolemia and atherosclerosis without consuming propiothiouracil,18 which is similar to the response in rabbits15 and miniature swine.18 These mice exhibit the same features of hypercholesterolemia associated with atherosclerosis development as the other animal models.

Lipoprotein alterations that occur in the mice during cholesterol feeding are similar to those in other animal models with some notable exceptions. On the control diet, both the C57BR/cdJ and CBA/J mice had HDL with an a-mobility similar to human HDL. There were no detectable a-migrating lipoproteins in the LDL fraction. Other mouse strains, such as CE/J, C3H/HeJ, and Swiss mice, have HDL with slightly faster electrophoretic mobility than human HDL and have LDL fractions that contain considerable a-migrating lipoprotein.19,20 When VLDL was present in the control animals, this lipoprotein had prebeta mobility similar to that of mice from strains used in other studies.20 During cholesterol feeding, a dramatic accumulation of VLDL, IDL, and LDL took place. There was relatively poor discrimination among these density fractions when they were subjected to agarose gel electrophoresis. While other animal models accumulate beta-VLDL, which is rich in cholesteryl ester and apolipoprotein E, the mouse VLDL retains prebeta mobility, although it also is enriched in cholesteryl ester and apo E. The IDL and LDL had prebeta and beta mobility and were distinctly smaller than VLDL, as evidenced by gel filtration chromatography. Thus, it would appear that cholesteryl ester-rich particles with beta mobility accumulate primarily in IDL and LDL fractions in mice of these strains.
Figure 8. Isoelectric focusing of HDL apolipoproteins. Conditions are as described in Figure 7. 1. HDL of resistant (CBA/J) mice; 2. HDL of susceptible (C57BR/6J) mice on the atherogenic diet; 3. mixture of HDL from susceptible and resistant mice. The mixture gives four bands in region c. Two of the major bands from each species overlap in the mixture.

The apolipoprotein composition of the lipoproteins agreed with other reports on mouse strains. The major component in HDL had a molecular weight comparable to that of human apo A-I. The isoelectric point, however, appeared higher than that of human apo A-I, which is readily resolved from apo E by isoelectric focusing. Furthermore, the isomorphs of this apolipoprotein were distinct in the two strains. Comparable variability in the composition of isomorphs of mouse apo A-I has been reported by others. Apo E is a minor constituent of HDL and does not accumulate extensively in HDL during cholesterol feeding.

The present observations about the inbred mice were similar to the findings with cholesterol-fed dogs. These latter have led to the recognition of hyperresponders (total plasma cholesterol of 350 mg/dl to 750 mg/dl) which fail to develop atherosclerosis and hyperresponders (total plasma cholesterol above 750 mg/dl) which develop atherosclerosis. The susceptible mice and the hyperresponding dogs have two striking features in common: the occurrence of the cholesteryl ester-rich VLDL and IDL, and a decrease in the plasma concentration of typical HDL. It is not known, however, whether or not the hyperresponding dogs also have a low phosphatidylcholine/free cholesterol ratio in their VLDL, IDL, and HDL fractions. The role of the increased total VLDL, IDL, and LDL fractions and the significance of the apoprotein composition in atherosclerosis development is open to speculation, as is the role of the lowered phosphatidylcholine/free cholesterol ratio in these lipoprotein fractions.

Recently, Morrisett et al. reported a higher apo E/total lipoprotein ratio in the d = 1.219 g/ml supernatant fraction from resistant mice and have called attention to the potential protective function of apo E. Innerarity et al. showed that cholesterol-induced beta VLDL caused massive accumulation of cholesteryl esters in mouse peritoneal macrophages in vitro, while HDL removed cholesterol from these cells. Obviously the balance between these lipoprotein fractions may be decisive in the tissue infiltration of the cholesteryl esters if similar mechanisms operate in cells other than the peripheral macrophages. Clearly, HDL saturated with free cholesterol would be less effective in this function. VLDL, IDL, and LDL that is saturated with free cholesterol could contribute to decreased stability and increased tissue infiltration by these particles, as discussed previously.

Several other explanations may account for the differences between the two strains of mice in developing atherosclerosis on the atherogenic diet. Roberts and Thompson have shown that the differences in cholesterol elevation are inherited. The present and the previous study show that there may be an inherited difference in the ability to handle exogenous cholesterol or to synthesize endogenous cholesterol and phosphatidylcholine. The susceptible strain actually gains less weight than the resistant strain, and is considerably more active and excitable with a higher basal metabolic rate than the resistant strain. It is possible, of course, that the susceptible strain may possess some basic weakness in the aortic wall and sinuses which eventually leads to atherosclerosis and is totally independent of the accumulation of any plasma lipoproteins of any chemical composition. Since no such weakness has been identified, the increased cholesterol saturation of all the plasma lipoproteins of the susceptible strain is now the only rational explanation of the induction and rapid progress of atherosclerosis in the inbred mice of susceptible strain.

Acknowledgments

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Lipoprotein levels in genetically selected mice with increased susceptibility to atherosclerosis.

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