Characterization of plasma lipoproteins of grain- and cholesterol-fed White Carneau and Show Racer pigeons

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Abstract Plasma cholesterol concentrations from White Carneau (WC) and Show Racer (SR) pigeons consuming a cholesterol-free grain diet averaged about 300 mg/dl, approximately 200 mg/dl as high density lipoproteins (HDL) and the remainder as low density lipoproteins (LDL). Consumption of a cholesterol-containing diet increased plasma cholesterol concentrations in both breeds to greater than 2000 mg/dl. Approximately one-half of this increase was as LDL with the remainder as beta-migrating very low density lipoproteins (β-VLDL). There was little change in HDL concentration. LDL from cholesterol-fed animals had a greater net negative charge than control LDL, and was larger (M_r = 10 × 10^6 vs 3.2 × 10^6) due to an increase in the number of cholesteryl ester molecules per particle. The principal apoprotein of LDL was apoB-100 with smaller amounts of apoA-I and several minor unidentified apoproteins. β-VLDL was cholesteryl ester-rich, could be separated into two size populations by gel chromatography, and contained apoB-100 as its principal apoprotein. Apoprotein E was not detected in any of the plasma lipoproteins. HDL from control and cholesterol-fed animals was composed of a single class of particles with virtually identical composition resembling HDL_2. The major apoprotein of HDL was apoA-I. There were no consistent quantitative or qualitative differences in the lipoproteins of the two breeds of pigeons that could help to explain the susceptibility to atherosclerosis of the WC or the resistance of the SR. — **Barakat, H. A., and R. W. St. Clair.** Characterization of plasma lipoproteins of grain- and cholesterol-fed White Carneau and Show Racer pigeons. *J. Lipid Res.* 1985. **26:** 1252–1268.

Supplementary key words atherosclerosis • hypercholesterolemia • VLDL-II • β-VLDL estrogen-induced lipoprotein changes • apoE • apoA-I • apoB-100 • apolipoproteins

It has long been recognized that certain breeds of pigeons (White Carneau [WC], Silver King) are susceptible to atherosclerosis, whereas other breeds (Show Racer [SR], Racing Homer) are resistant, even though they consume the same cholesterol-free grain diet and are not different with respect to plasma cholesterol concentration (1, 2). An important feature of pigeon atherosclerosis is that it develops spontaneously (2). While consuming a cholesterol-free grain diet, pigeons are hypercholesterolemic relative to most animals, with the bulk of their plasma cholesterol transported as high density lipoproteins (HDL). When fed a cholesterol-rich diet, plasma cholesterol concentrations rise rapidly and the rate of development of atherosclerosis is accelerated and the severity and extensiveness of atherosclerosis is enhanced. Nevertheless, the difference in susceptibility between the WC and SR breeds is maintained. Although differences in several metabolic parameters have been described in arterial tissue from atherosclerosis-susceptible and resistant breeds and strains of pigeons (for recent reviews see references 3 and 4), no specific cause-and-effect relationships have been identified.

Differences in the concentration and composition of plasma lipoproteins are known to influence the development of atherosclerosis in human beings and a number of animal species. Studies from our laboratory and others have shown that hypercholesterolemic serum and low density lipoproteins (LDL) isolated from hypercholesterolemic monkeys enhance cholesterol accumulation in cells in culture (5–9). LDL from hypercholesterolemic plasma has been shown to be larger and contain an increased number of cholesteryl ester molecules per LDL particle (7, 10–12). In a variety of species, including dogs, rats, rabbits, swine, monkeys, and pigeons (3, 13), feeding a cholesterol-rich diet can result in the accumulation of a β-migrating, cholesteryl ester-rich very low density lipoprotein (β-VLDL) that has been reported (14, 15) to be associated with development of atherosclerosis. The association of β-VLDL with accelerated atherosclerosis may be related to its ability to promote cholesteryl ester

Abbreviations: LDL, low density lipoproteins; HDL, high density lipoproteins; β-VLDL, beta-migrating very low density lipoproteins; VLDL, very low density lipoproteins; WC, White Carneau; SR, Show Racer; SDS, sodium dodecyl sulfate; SAA, serum amyloid protein.

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accumulation in macrophages (for recent review see ref. 16). Thus, it appears that feeding a cholesterol-rich diet not only alters the composition of LDL but also causes the elaboration of a new lipoprotein species such as β-VLDL. Such alterations may be of importance in promoting cholesteryl ester accumulation in cells and the subsequent development of atherosclerosis.

Although there are some limited compositional data and some information on electrophoretic mobility of the lipoproteins of pigeons (3, 17–19), there has been no comprehensive study of the plasma lipoproteins of normal or cholesterol-fed pigeons. With the exception of apolipoprotein A-1 from normal WC pigeons (20), there is also little published information on pigeon apolipoproteins. Hence, this study was undertaken in order to determine whether there are differences in the composition or concentration of plasma lipoproteins from grain-fed and cholesterol-fed WC and SR pigeons that might correlate with the known differences in susceptibility to atherosclerosis of these breeds. In this report we present the data on the composition and concentration of the plasma lipoproteins. In a subsequent report we will present data on the relationship between plasma lipoprotein concentration and composition and the extent and severity of coronary artery and aortic atherosclerosis.

METHODS

Animals and experimental plan

White Carneau pigeons, 5–6 months of age, and Show Racer pigeons, 3–6 months of age, (30 of each breed) were obtained from our breeding colonies and were fed a cholesterol-free pelleted grain diet (Davis Complete Pigeon Ration, W. A. Davis Milling Co., High Point, NC) for 1 month prior to starting the experimental diets. The analysis of this diet as supplied by the W. A. Davis Milling Co. is 15% crude protein, 2.5% crude fat, and 6% crude fiber. At this time 18 animals from each breed were randomly assigned to a dietary regimen of pigeon pellets to which cholesterol and lard were added to a final concentration of 0.5% cholesterol (w/w) and 10% lard (w/w). The remaining 12 animals of each breed continued to consume the pigeon pellets alone and served as controls. At monthly intervals for 6 months animals were fasted overnight and blood was drawn from each animal into tubes containing EDTA (1 mg/ml). The tubes were chilled on ice and the plasma was prepared immediately. Plasma cholesterol, triglyceride, and HDL-cholesterol concentrations were determined as described previously (21, 22). Eight ml of blood was drawn from each animal after the second and fifth month of consuming the experimental diets in order to carry out a detailed analysis of lipoprotein concentration and composition. At the termination of the experimental period (6 months) all animals were killed with an overdose of sodium pentabarbital and the degree and severity of coronary artery and aortic atherosclerosis were assessed. Samples of liver and spleen were taken for transmission electron microscopy and other samples were analyzed for cholesteryl ester content. Data on extent of atherosclerosis, tissue cholesterol content, and ultrastructural appearance will be presented in a separate publication.

Isolation and characterization of plasma lipoproteins

For the cholesterol-fed animals, the plasma lipoproteins were isolated individually from each animal in the study. For control animals, the lipoproteins were isolated from individual animals but their composition was determined on pooled samples prepared by combining equivalent amounts of plasma from each animal into a single pooled sample. This was done to assure sufficient material for analysis. All analyses were done in duplicate.

For separation of lipoprotein fractions, 2 ml of plasma from individual animals was added to Beckman Ultraclear centrifuge tubes and overlayered with at least 3 ml of a 1.006 g/ml NaCl solution containing 0.01% EDTA. After centrifugation at 40,000 rpm at 15°C for 18 hr in a Beckman 40.2 rotor using a Beckman L2-65B or L5-50 ultracentrifuge, the lipoproteins with density (d) < 1.006 g/ml were isolated in the top 2 ml by tube slicing. The $d < 1.006$ g/ml lipoproteins were adjusted to a known volume and aliquots were taken for total cholesterol and triglyceride content, agarose electrophoresis (23), and for chemical composition. The remainder of the $d < 1.006$ g/ml fractions was concentrated approximately 2- to 5-fold by dialysis against dry dextran T-500 (Pharmacia) and separated by chromatography on 2% agarose (Bio-Gel A-50 m). The lipoproteins were eluted with a high salt buffer containing 0.3 M NaCl, 0.1 M KH_2PO_4, 0.01% EDTA, pH 7.4. The high salt buffer was beneficial in minimizing aggregation of lipoproteins, particularly those from cholesterol-fed animals. Fractions were pooled as described, concentrated, and analyzed.

An aliquot of the $d > 1.006$ g/ml fraction containing LDL and HDL was analyzed for total cholesterol and triglyceride and for HDL cholesterol content. In this way LDL cholesterol could be calculated as total plasma cholesterol minus $d < 1.006$ g/ml cholesterol minus HDL cholesterol.

The $d > 1.006$ g/ml fraction was adjusted to 1.225 g/ml with solid KBr, overlayered with 3 ml of a 1.215 g/ml KBr solution containing 0.01% EDTA, and centrifuged in a Beckman SW-40 rotor at 36,000 rpm for 40 hr at 15°C. The density 1.006–1.215 g/ml lipoproteins were isolated in the top 2 ml by tube slicing. The lipoproteins in this fraction were separated by chromatography on 4% agarose (Bio-Gel A-15m) as described previously (6). LDL and

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HDL fractions were concentrated by dialysis and aliquots were analyzed for chemical composition, agarose gel electrophoresis, and for apoproteins. The molecular weight of the LDL from individual birds was estimated at month 5 only, by including trace amounts of a known molecular weight ^{125}I-labeled LDL internal standard in the separation of the d 1.006–1.215 g/ml lipoproteins on the 4% agarose column (10).

HDL isolated by agarose column chromatography was concentrated by dialysis to approximately 1 mg of protein/ml and adjusted to density 1.125 g/ml with solid KBr. The HDL was separated by density gradient centrifugation at 36,000 rpm for 48 hr in an SW-40 rotor at 15°C. The gradient was prepared as follows. Four ml of d 1.21 g/ml solution was placed in the bottom of the centrifuge tube. This was overlayered with 6 ml of the HDL solution at d 1.125 g/ml and finally with 3 ml of d 1.050 g/ml solution. All density solutions contained 0.9% NaCl, 0.01% EDTA, and the amount of KBr necessary to achieve the indicated density. Following centrifugation the bottom of the tube was pierced and fractions of 0.4 ml were collected by pumping Fluorinert (ISCO, Inc.), d 1.85 g/ml, into the bottom of the tube and collecting samples from the top of the gradient. The elution of the protein peaks was monitored continuously by absorbance using a Buchler Fractoscan UV monitor. Densities of individual fractions were determined by measuring refractive index. Tubes containing no lipoproteins were also run to monitor the gradient.

The chemical composition of β-VLDL, LDL, and HDL was determined as described previously (5). Compositional parameters included total cholesterol, free and esterified cholesterol, protein, phospholipid, and triglyceride.

Apoproteins from isolated lipoproteins were separated by electrophoresis in horizontal slab linear gradients of 4 to 30% polyacrylamide with a 4% polyacrylamide stacking gel. Buffers were those described by Laemmli (24) and contained 0.1% sodium dodecyl sulfate (SDS). Lipoproteins were lyophilized and then resolubilized in 0.46% barbital, 5% mercaptoethanol, 3% SDS, 0.001% bromphenol blue, pH 8.3, at 100°C for 2 min prior to application to the gradient gel. Forty μg of protein was applied to each lane of the gel.

RESULTS

Plasma cholesterol concentrations of both breeds of pigeons rose dramatically when they consumed the cholesterol-containing diet (**Fig. 1**) and remained elevated throughout the 6 months of cholesterol consumption. In SR pigeons the cholesterol concentration increased through the third month reaching a level approximately 500 mg/dl higher than for the WC. This differential was

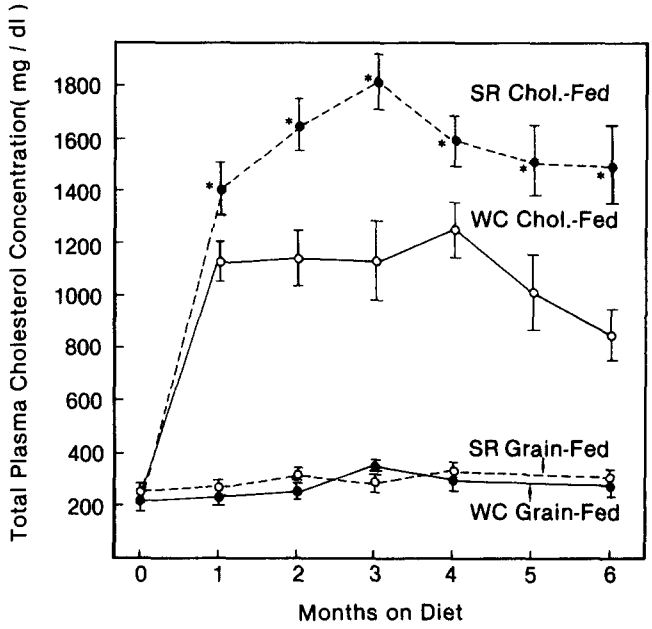

Fig. 1. Plasma cholesterol concentration as a function of time and diet. Results are the mean ± SEM of the following number of animals in each group: SR cholesterol-fed N = 16, WC cholesterol-fed N = 17, SR grain-fed N = 12, WC grain-fed N = 12. The * indicates those points that are significantly different ($P < 0.05$) from the WC cholesterol-fed group using a two-tailed Student's *t*-test.

maintained throughout the duration of the experiment. In contrast, plasma cholesterol levels of the grain-fed pigeons of both breeds were not significantly different, and there was little change with time. The higher plasma cholesterol concentration of cholesterol-fed SR relative to WC was unexpected, and was different from our previous studies in which the plasma cholesterol response was similar for both breeds (3).

The distribution of cholesterol among the lipoproteins of grain-fed and cholesterol-fed pigeons was determined after 2 and 5 months of consuming the respective diets. In the grain-fed birds (**Table 1**) plasma cholesterol concentrations in the WC pigeons were slightly higher than in the SR at 2 months but similar after 5 months in both sexes. The relative distribution of cholesterol in the d < 1.006 g/ml fraction (VLDL), LDL, and HDL was similar in both breeds at the two time periods. VLDL concentrations were extremely low, ranging from approximately 3% of total plasma cholesterol to amounts too small to detect. Seventy-five to 80% of the cholesterol was transported as HDL with the remaining 20–25% as LDL. At 2 months on the experiment none of the birds were in the egg-laying phase, while after 5 months many of the females were laying eggs. The 5-month time period correlated with the time of year of most active reproduction. This was accompanied by a marked increase in plasma triglyceride concentrations at 5 months in females only. This occurred in both breeds. There was also an increase

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TABLE 1. Distribution of plasma cholesterol among the lipoproteins of grain-fed pigeons

	White Carneau		Show Racer	
	2 months	5 months	2 months	5 months
	mg/dl ± SEM			
Total plasma cholesterol				
All animals	323 ± 11 (12)[a]	302 ± 15 (12)	253 ± 11 (12)	281 ± 20 (12)
Males	331 ± 8 (5)	306 ± 10 (5)	252 ± 9 (8)	255 ± 11 (8)
Females	318 ± 16 (7)	300 ± 27 (7)	255 ± 33 (4)	333 ± 55 (4)
d < 1.006 g/ml cholesterol				
All animals	11 ± 2 (12)	ND[b]	9 ± 2 (12)	ND
Males	12 ± 2 (5)	ND	8 ± 3 (8)	ND
Females	10 ± 2 (7)	ND	12 ± 2 (4)	ND
LDL cholesterol				
All animals	59 ± 7 (12)	145 ± 27 (12)	39 ± 5 (12)	108 ± 27 (12)
Males	47 ± 11 (5)	76 ± 7 (5)	35 ± 5 (8)	57 ± 8 (8)
Females	67 ± 10 (7)	195 ± 36 (7)	50 ± 6 (4)	212 ± 46 (4)
HDL cholesterol				
All animals	254 ± 7 (12)	157 ± 21 (12)	198 ± 13 (12)	173 ± 15 (12)
Males	264 ± 9 (5)	231 ± 3 (5)	207 ± 6 (8)	198 ± 7 (8)
Females	246 ± 10 (7)	105 ± 14 (7)	178 ± 43 (4)	121 ± 32 (4)
Total plasma triglyceride				
All animals	93 ± 5 (12)	667 ± 238 (12)	101 ± 7 (12)	452 ± 247 (12)
Males	91 ± 10 (5)	64 ± 3 (5)	99 ± 7 (8)	76 ± 7 (8)
Females	94 ± 7 (7)	1097 ± 323 (7)	104 ± 18 (4)	1204 ± 666 (4)

[a]Values in parentheses indicate the number of animals per group.
[b]ND, not determined.

in LDL cholesterol and a decrease in HDL cholesterol in the egg-laying females of both breeds at 5 months. These changes offset one another resulting in no net change in total plasma cholesterol concentration between months 2 and 5. In males, there was a small increase in LDL cholesterol at 5 months but no difference in HDL cholesterol between 2 and 5 months.

Cholesterol feeding produced a variety of changes in cholesterol distribution among the plasma lipoproteins (**Table 2**). There was a marked increase in the d < 1.006 g/ml fraction in both breeds. Approximately 25% of the total plasma cholesterol of the WC pigeons was transported by this fraction, while in the SR up to 50% of the total plasma cholesterol was transported as d < 1.006 g/ml lipoproteins. With the exception of the 2-month SR group, there was a suggestion of a higher concentration of d < 1.006 g/ml lipoprotein cholesterol in males which correlated with a slightly higher average total plasma cholesterol concentration. Approximately 50% of the total plasma cholesterol of the cholesterol-fed animals was transported as LDL. There were no major differences between the sexes in the proportion of cholesterol transported among specific lipoprotein fractions. The cholesterol-fed birds had LDL cholesterol concentrations that were 5–10 times higher than grain-fed animals. HDL cholesterol concentration in the cholesterol-fed birds did not differ from the grain-fed animals and were not different between the sexes. Thus, the additional plasma cholesterol that resulted from cholesterol feeding was transported by both LDL and the d < 1.006 g/ml lipoproteins. Plasma triglyceride concentrations were not influenced by cholesterol feeding as can be seen in males or non-egg-laying females (2 months). The increased triglyceride concentration of females seen at 5 months was associated with their egg-laying cycle and was observed in the cholesterol-fed birds as well, although the extent of hypertriglyceridemia was less than for grain-fed birds.

Plasma triglycerides of the animals fed cholesterol for 5 months were transported in approximately equivalent amounts by LDL and the d < 1.006 g/ml lipoproteins (**Table 3**). This proportion was unchanged regardless of whether the pigeons were hypertriglyceridemic females or males with normal triglyceride concentrations. Similar comparisons were not made for grain-fed animals. Plasma triglycerides of SR pigeons at 5 months were only slightly higher than for males, owing presumably to fewer birds in this group being in the egg-laying cycle at the time of lipoprotein analysis.

The chemical composition of the isolated d < 1.006 g/ml lipoproteins is shown in **Table 4.** The d < 1.006 g/ml fraction from cholesterol-fed animals was rich in cholesteryl esters. The composition changed dramatically, however, in egg-laying females. Whereas in males the content of triglyceride was low (<5% of mass), in egg-laying females the triglyceride content increased dramatically to as much as 50% of the total mass. Because

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TABLE 2. Distribution of plasma cholesterol among the lipoproteins of cholesterol-fed pigeons

	White Carneau		Show Racer	
	2 months	5 months	2 months	5 months
	mg/dl ± SEM			
Total plasma cholesterol				
All animals	1146 ± 98 (18)[a]	1013 ± 148 (17)	1645 ± 109 (16)	1518 ± 135 (16)
Males	1288 ± 164 (8)	1278 ± 291 (8)	1626 ± 240 (5)	1629 ± 181 (5)
Females	1032 ± 120 (10)	778 ± 80 (9)	1653 ± 132 (11)	1467 ± 181 (11)
d < 1.006 g/ml cholesterol				
All animals	254 ± 67 (18)	251 ± 68 (17)	811 ± 99 (16)	466 ± 71 (16)
Males	371 ± 134 (8)	385 ± 123 (8)	796 ± 210 (5)	596 ± 142 (5)
Females	161 ± 55 (10)	131 ± 55 (9)	816 ± 120 (11)	408 ± 83 (11)
LDL cholesterol				
All animals	605 ± 52 (18)	555 ± 89 (17)	607 ± 32 (16)	804 ± 94 (16)
Males	634 ± 70 (8)	656 ± 169 (8)	582 ± 67 (5)	792 ± 85 (5)
Females	582 ± 80 (10)	440 ± 63 (9)	618 ± 38 (11)	809 ± 138 (11)
HDL cholesterol				
All animals	287 ± 10 (18)	228 ± 18 (17)	227 ± 13 (16)	251 ± 10 (16)
Males	284 ± 21 (8)	238 ± 13 (8)	244 ± 22 (5)	242 ± 15 (5)
Females	290 ± 9 (10)	216 ± 41 (9)	219 ± 17 (11)	256 ± 14 (11)
Total plasma triglyceride				
All animals	87 ± 8 (18)	416 ± 195 (17)	115 ± 12 (16)	155 ± 32 (16)
Males	100 ± 15 (8)	130 ± 30 (8)	125 ± 31 (5)	116 ± 48 (5)
Females	77 ± 7 (10)	754 ± 354 (9)	111 ± 13 (11)	172 ± 43 (11)

[a]Values in parentheses indicate the number of animals per group.

these values represent the mean of all females, some of which were not in their egg-laying cycle (as evidenced by the SR females), the amount of triglyceride in the d < 1.006 g/ml fraction is probably much higher than 50% of total mass for egg-laying animals only. Since we have no data on the particular phase of the egg-laying cycle or the influence of the phase of the cycle on triglyceride concentrations, it was not possible to determine maximum triglyceride content of these lipoproteins. Nevertheless, regardless of the composition of the core lipids (cholesteryl esters or triglycerides), the proportion of surface components (protein, phospholipid, and free cholesterol) remained relatively constant at between 40–50% of total mass. Furthermore, under conditions of hypertriglyceridemia, the triglycerides appear to replace cholesteryl esters in the core of the lipoprotein so that the sum of the core components remains constant.

Since there were extremely low concentrations of VLDL (d < 1.006 g/ml) in grain-fed birds, no data on the composition of this fraction were obtained.

After removal of the d < 1.006 g/ml lipoproteins, the lipoproteins within the density fraction 1.006–1.21 g/ml were concentrated by flotation in the ultracentrifuge at 1.21 g/ml and separated by agarose column chromatography. Typical chromatograms for grain- and cholesterol-fed pigeons are shown in **Fig. 2.** Two major peaks were seen, corresponding to LDL and HDL. The LDL peak from the grain-fed birds eluted at approximately the same location as the monkey ^{125}I-labeled LDL internal standard, while the LDL from cholesterol-fed birds eluted earlier and as a result was larger than the LDL internal standard.

Composition of the LDL was determined from individual cholesterol-fed animals and on the pooled frac-

TABLE 3. Distribution of triglycerides among the lipoproteins of cholesterol-fed birds sampled at 5 months

	White Carneau	Show Racer
	5 months	5 months
	mg/dl ± SEM	
Total plasma triglyceride[a]		
All animals	461 ± 195 (17)[b]	155 ± 32 (16)
Males	130 ± 30 (8)	116 ± 48 (5)
Females	754 ± 354 (9)	172 ± 43 (11)
d < 1.006 g/ml triglyceride		
All animals	212 ± 103 (17)	70 ± 15 (16)
Males	50 ± 19 (8)	64 ± 20 (5)
Females	356 ± 190 (7)	72 ± 22 (11)
LDL triglyceride		
All animals	205 ± 86 (17)	60 ± 13 (16)
Males	45 ± 17 (8)	36 ± 19 (5)
Females	347 ± 152 (9)	71 ± 17 (11)
HDL triglyceride		
All animals	50 ± 10 (17)	34 ± 4 (16)
Males	39 ± 10 (8)	32 ± 5 (5)
Females	59 ± 18 (9)	35 ± 6 (11)

[a]Data also included in Table 2.
[b]Data are available only for cholesterol-fed animals sampled at 5 months. Values in parentheses indicate the number of animals per group.

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TABLE 4. Percent composition of β-VLDL from cholesterol-fed pigeons after 5 months of consuming the experimental diet

	White Carneau	Show Racer
Protein	11.0 (17)[a]	11.1 (16)
Males	10.5 (8)[b]	8.8 (5)
Females	11.5 (9)	12.2 (11)
Phospholipid	19.2	16.1
Males	16.5	15.3
Females	21.6	16.9
Cholesteryl ester	32.0	53.3
Males	55.2	57.5
Females	11.6	51.4
Free cholesterol	9.4	12.1
Males	12.7	13.7
Females	6.4	11.3
Triglyceride	28.2	7.1
Males	5.0	4.6
Females	48.9	8.2

[a]Values in parentheses indicate the number of animals whose plasma was pooled prior to analysis. The same plasma pool was used for analyses of all of the above parameters. Since pooled plasma was used for analysis it was not possible to evaluate variability among individual animals statistically.

[b]The d < 1.006 g/ml fractions were pooled according to the sex of the birds and analyzed as pooled samples.

tions from grain-fed animals (**Table 5**). With the exception of slightly lower free cholesterol and higher triglyceride concentrations, the composition of LDL from grain-fed WC and SR pigeons (males and non-egg-laying females) was similar to that described for LDL from a variety of mammals including man (25) and nonhuman primates (26, 27). In egg-laying females there was a decrease in the percentage composition of cholesteryl esters and an increase in triglycerides. Upon cholesterol feeding there was an increase in the proportion of cholesteryl esters, a decrease in triglyceride, a decrease in protein, and a small increase in free cholesterol. Similar changes occurred in the LDL from WC and SR pigeons. The increase in triglyceride content in the d < 1.006 g/ml lipoproteins observed in female animals (both grain- and cholesterol-fed) was not as obvious in the LDL from cholesterol-fed animals.

The molecular weight of LDL from the grain-fed male birds was approximately 3.2×10^6 for both WC and SR (**Table 6**). The LDL from females was larger with average molecular weights reaching 5.7×10^6. This is consistent with the enrichment of these LDL with triglycerides. Cholesterol feeding had a marked influence on LDL molecular weight with average molecular weights as high as 9.9×10^6 for cholesterol-fed male SR pigeons (Table 6).

The change in LDL molecular weight was positively correlated with total plasma cholesterol concentrations (**Fig. 3**). Although the distribution of individual animals in Fig. 2 may suggest the existence of several discrete populations, rather than a continuum indicated by the linear regression, there was no evidence that LDL from WC or SR pigeons were different in this regard.

The number of component molecules per LDL particle is shown for cholesterol-fed WC (**Fig. 4A**) and SR (Fig. 4B) pigeons. These data were calculated from the LDL molecular weights and the percent composition and molecular weights (see legend of Fig. 4) of the component parts of the LDL particles. LDL from male grain-fed WC and SR pigeons (see tabular inserts in Fig. 4) averaged: protein, 7.8; cholesterol, 800; cholesteryl ester, 1195; phospholipid, 895; and triglyceride, 475 molecules/LDL particle. In egg-laying females there was an increase in the number of triglyceride molecules (2780 molecules/particle) at the expense of cholesteryl esters that decreased to approximately 723 molecules/particle. There was little difference in the proportion of molecular components of LDL from grain-fed WC or SR pigeons. Upon cholesterol feeding there was an increase in the number of cholesteryl ester molecules to approximately 10,000 molecules/particle for the largest LDL studied ($M_r = 13.4 \times 10^6$). Increases in the number of molecules per particle with increasing molecular weight were also seen for the other components of LDL, but not to the extent of that seen for cholesteryl esters. As a result of their higher plasma cholesterol con-

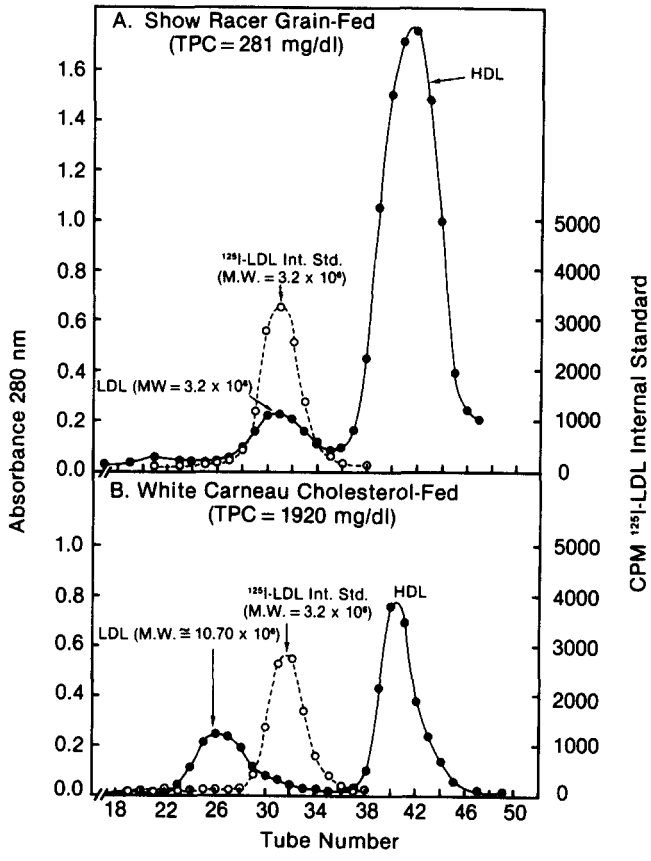

Fig. 2. Typical elution pattern from 4% agarose gel, of d > 1.006 g/ml lipoproteins from grain-fed and cholesterol-fed pigeons. TPC, total plasma cholesterol concentration.

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TABLE 5. Percent composition of low density lipoproteins of grain- and cholesterol-fed pigeons at 5 months

	Protein	Phospholipid	Free Chol.	Chol. Esters	Triglyceride
Grain-fed[a]					
White Carneau					
All animals	21.9 (12)[b]	22.1	5.9	14.7	35.4
Males	31.2 (5)	18.7	10.4	22.9	16.8
Females	15.3 (7)	24.5	2.7	8.8	48.6
Show Racer					
All animals	25.9 (12)	24.5	6.6	21.0	22.1
Males	30.1 (4)	24.5	8.9	26.7	9.8
Females	17.4 (8)	24.6	1.9	9.5	46.6
Cholesterol-fed					
White Carneau					
All animals	13.5 ± 1.2 (17)	24.2 ± 1.6	11.4 ± 0.6	40.8 ± 2.7	10.2 ± 2.7
Males	12.4 ± 2.0 (8)	21.8 ± 1.1	12.3 ± 0.6	44.9 ± 3.1	8.9 ± 2.5
Females	14.6 ± 1.4 (9)	26.6 ± 2.7	10.4 ± 0.9	36.8 ± 0.4	11.5 ± 4.8
Show Racer					
All animals	9.5 ± 0.4 (16)	22.0 ± 0.8	12.7 ± 0.3	52.1 ± 1.1	3.7 ± 0.7
Males	10.1 ± 0.6 (5)	20.6 ± 0.8	12.3 ± 1.0	54.5 ± 0.7	2.6 ± 0.6
Females	9.2 ± 0.6 (11)	22.6 ± 1.0	14.0 ± 0.3	51.5 ± 1.4	4.1 ± 0.9

[a]Values for the grain-fed birds are from the duplicate analysis of the pooled plasma obtained as described in the legend to Table 4.
[b]Values in parentheses represent the number of animals per group and apply for all parameters indicated. For cholesterol-fed animals the results are the mean ± SEM for the analysis of individual animals.

centration, on the average, SR pigeons had larger LDL than the WC pigeons. However, the changes in the molecular components for a comparable size LDL particle were virtually identical, suggesting that there was no basic physical-chemical difference between the LDL of the two breeds.

Agarose electrophoresis patterns of whole plasma, the d > 1.006 g/ml and d < 1.006 g/ml fractions, and LDL isolated by agarose gel chromatography are shown in **Fig. 5.** Representative examples from grain-fed and cholesterol-fed pigeons are included. With animals of similar plasma cholesterol concentration there was no systematic difference in electrophoretic mobility of lipoproteins between WC and SR pigeons. Whole plasma from grain-fed birds had two lipoprotein bands. One migrated in the same relative position as mammalian HDL and the other remained at or near the origin. There was no detectable VLDL in the whole plasma or in the d < 1.006 g/ml fraction. The same two fractions seen in whole plasma were present in the d > 1.006 g/ml fraction. LDL from grain-fed animals separated by agarose column chromatography also remained at the origin upon agarose gel electrophoresis. Whole plasma from cholesterol-fed animals also showed two lipoprotein bands by electrophoresis. One was alpha-migrating and had an electrophoretic mobility similar to HDL from grain-fed animals. The other had beta-mobility and was clearly different than LDL from grain-fed birds. When the whole plasma was fractionated by ultracentrifugation at d 1.006 g/ml, the beta-migrating band from cholesterol-fed animals was shown to contain two major lipoproteins. One was isolated in the d < 1.006 g/ml fraction and the other in the d > 1.006 g/ml fraction. The LDL isolated by agarose column chromatography from cholesterol-fed animals had the same beta-mobility as the LDL fraction from the d > 1.006 g/ml fraction of plasma. Thus, the beta-migrating lipoproteins of the cholesterol-fed animals contained both a cholesterol-rich VLDL (β-VLDL) and

TABLE 6. Average plasma cholesterol concentrations and LDL molecular weights of grain- and cholesterol-fed pigeons at 5 months

	Plasma Cholesterol	LDL Mol. Wt.
	mg/dl	$\times 10^{-6}$
Grain-fed		
White Carneau		
All animals	302 ± 15 (12)[a]	4.14[b]
Males	306 ± 10 (5)	3.22
Females	300 ± 27 (7)	4.80
Show Racer		
All animals	281 ± 20 (12)	4.03
Males	255 ± 11 (8)	3.20
Females	333 ± 55 (4)	5.70
Cholesterol-fed		
White Carneau		
All animals	1013 ± 148 (17)	5.44 ± 0.50
Males	1278 ± 291 (8)	6.36 ± 0.87
Females	778 ± 80 (9)	4.52 ± 0.31
Show Racer		
All animals	1518 ± 135 (16)	8.41 ± 0.61
Males	1629 ± 181 (5)	9.91 ± 1.16
Females	1567 ± 181 (11)	7.74 ± 0.67

[a]Values in parentheses represent the number of animals per group and apply for all parameters indicated. Results are the mean ± SEM.
[b]LDL molecular weights of the grain-fed birds were determined from the pooled plasma obtained as described in the legend to Table 4.

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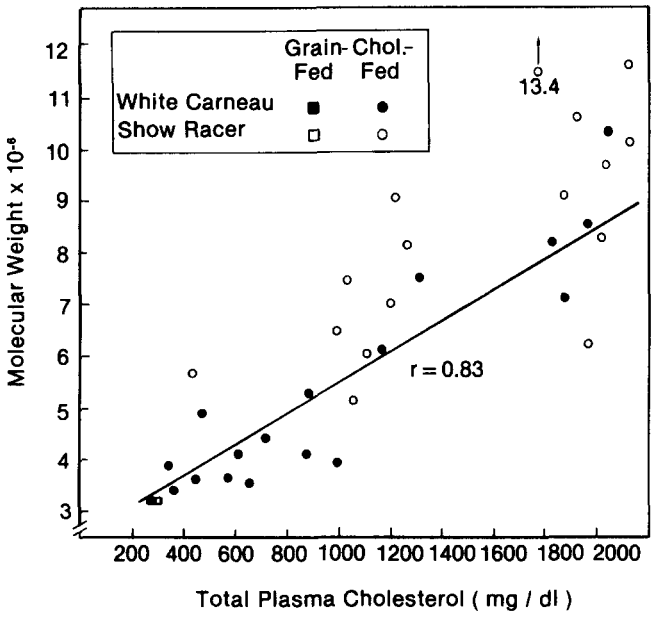

Fig. 3. Relationship of LDL molecular weight to total plasma cholesterol concentration in grain-fed and cholesterol-fed pigeons. Values for grain-fed birds represent the mean of duplicate determinations on the pooled plasma from five male WC and eight male SR pigeons obtained after 6 months on experiment. All other values are from individual cholesterol-fed birds after 5 months on diet.

LDL. Furthermore, the LDL from the cholesterol-fed animals had a different electrophoretic mobility than LDL from grain-fed birds, suggesting a greater net negative change.

The difference in electrophoretic mobility of the LDL from the grain-fed and cholesterol-fed animals appeared to be a function of the extent of hypercholesterolemia (**Fig. 6**). Although in the examples shown in Fig. 6 there also appeared to be some change in the electrophoretic mobility of HDL with increasing plasma cholesterol concentrations, this was not a consistent finding. In female animals during the egg-laying phase (as indicated by the mild hypertriglyceridemia) there was a further change in the LDL/β-VLDL fraction in cholesterol-fed animals resulting in a net migration toward the cathode (Fig. 6D). A similar change in LDL was seen in grain-fed birds (data not shown).

Based on measurements of cholesterol in the d < 1.006 g/ml fraction of whole plasma, detectable concentrations of β-VLDL first appeared when plasma cholesterol concentrations exceeded 600–700 mg/dl. Since only a single beta-migrating lipoprotein was seen on electrophoresis, regardless of the plasma cholesterol concentration, the change in electrophoretic mobility with increasing extent of hypercholesterolemia must occur with both β-VLDL and LDL. At plasma cholesterol concentrations greater than about 1500 mg/dl, there was little further change in electrophoretic mobility of β-VLDL and LDL. The same changes occurred for WC and SR pigeons.

To further characterize β-VLDL we separated the d < 1.006 g/ml fraction from hypercholesterolemic pigeons by chromatography on 2% agarose (Bio-Gel A-50m). Typical elution patterns are shown in **Fig. 7.** As can be seen, there were two peaks. Greater than 90% of the cholesterol of the d < 1.006 g/ml fraction was isolated in peak II. Again, no obvious differences between WC and SR pigeons were seen in the elution pattern of the d < 1.006 g/ml fraction from the 2% agarose column.

The chemical composition of HDL, isolated by agarose column chromatography, is shown in **Table 7.** The composition of HDL was similar to that of mammalian HDL with approximately 45% of mass as protein, 30% phospholipid, 4% free cholesterol, 17% cholesteryl ester, and 4% triglyceride. HDL from grain-fed females had a slight increase in triglycerides while no such change occurred for HDL from cholesterol-fed females. Otherwise, there were no significant differences in the chemical composition of HDL from grain-fed or cholesterol-fed animals or between WC or SR pigeons.

To determine whether pigeon HDL existed in different density subclasses, such as HDL_2 and HDL_3, the HDL isolated by agarose column chromatography was subjected to density gradient ultracentrifugation and results are shown in **Fig. 8.** Only a single density class of HDL was detected in both grain-fed and cholesterol-fed birds. The average HDL density was 1.093 g/ml which is similar to that of mammalian HDL_2. There was no evidence of a lipoprotein of the density of HDL_3. No differences were found in the HDL density between WC and SR pigeons and, consistent with the lack of change in chemical composition (Table 7), there was no effect of cholesterol feeding on the density of HDL.

The apoproteins of the isolated lipoproteins were separated by gradient gel electrophoresis and are shown in Fig. 9–11. African green monkey lymph chylomicrons with apoproteins identified as indicated and commercially prepared molecular weight standards were run with each gel. β-VLDL from cholesterol-fed animals contained apoB-100 and apoA-I as the major apoproteins (**Fig. 9**). There were several unidentified proteins that migrated between the 30-K and 67-K standards. One had a molecular weight of approximately 67 K and the other 40 K. Another unidentified apoprotein with a molecular weight slightly less than that of serum amyloid protein (SAA) was also seen. Neither apoB-48 nor an apoprotein of the size of mammalian apoE was seen in β-VLDL. The β-VLDL from an egg-laying female (lane E) showed the presence of an additional protein band with a molecular weight of approximately 10–12 K and a relative decrease in apoA-I and the other unidentified apoproteins. There was no apparent reduction in apoB-100, however. There was not sufficient VLDL available from grain-fed animals to carry out apoprotein analysis.

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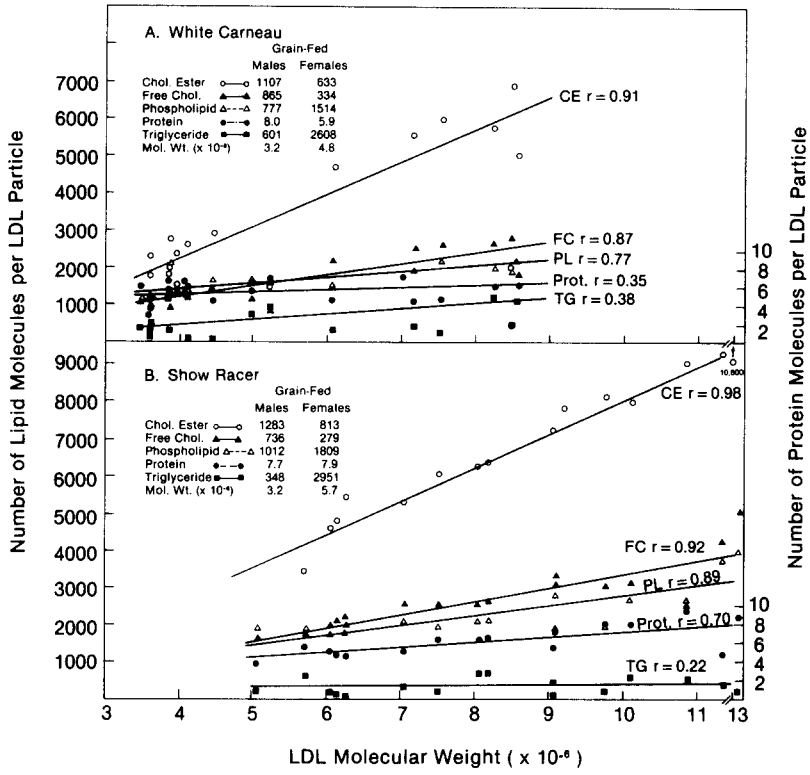

Fig. 4. Relationship of LDL molecular weight to the number of constituent molecules per particle. Molecules per particle of LDL were calculated from the LDL molecular weight and the percent composition and molecular weights of the component parts of the LDL particle. The following molecular weights were used for these calculations: free cholesterol (FC), 387; cholesteryl esters (CE), 660; triglycerides (TG), 900; and phospholipids (PL), 775; protein, 125,000. The number of FC, CE, TG, and PL molecules per LDL particle is indicated on the left vertical axis while the number of protein molecules is shown on the right vertical axis. Since the molecular weight of the major apoprotein of pigeon LDL (apoB) is not known, and other apoproteins such as A-1 are present in significant quantities in pigeon LDL, we selected a molecular weight (125,000) for the LDL protein since this molecular weight fell within the range of molecular weights of proteins present in the LDL. Thus, only comparisons of the relative change in LDL protein per particle should be made. The true value for the absolute number of protein molecules per particle cannot be calculated until the molecular weight of apoB is determined. Results for the number of constituent molecules for LDL from grain-fed animals of both breeds are shown in the tabular inserts. This was the same LDL as described in the legend to Fig. 3.

LDL apoproteins are shown in **Fig. 10.** In grain-fed animals apoB-100 was the major apoprotein. An apoB-48 band was not present even though there were multiple proteins that were somewhat smaller than monkey apoB-48. From these studies it is impossible to know whether these represent degradation products of apoB-100 (28) or new apoproteins specific for avian species such as the pigeon. ApoA-I and several unidentified proteins migrating between the 67-K and 43-K molecular weight standards were also present in grain-fed animals. The predominant apoproteins of LDL from cholesterol-fed animals were apoB-100 and apoA-I. These LDL also had a protein that was slightly larger than the 67-K standard and the appearance of a single major protein that was slightly larger than the 43-K standard, and thus resembled the size of mammalian apoA-IV (29). The 67-K protein was most likely not albumin since the LDL were purified by agarose gel chromatography before apoprotein analysis. The two proteins migrating between 43 and 67 K in the grain-fed animals were absent in the LDL from cholesterol-fed animals. Small amounts of an apoprotein with molecular weight less than that of SAA were seen. As with β-VLDL, no protein with a molecular weight of mammalian apoE was observed. In the LDL of egg-laying females (lanes D, F, and H), as was also true of β-VLDL, there was the appearance of a protein with molecular weight of approximately 10–12 K and a relative decrease in all of the other apoproteins except B-100. There were no consistent quantitative or qualitative differences in the apoproteins of LDL from WC or SR pigeons.

The apoproteins of LDL of different molecular weights are shown in **Fig. 11.** The LDL of grain-fed WC and SR (lanes F and K) had the same pattern of apoproteins, including the two unidentified proteins migrating be-

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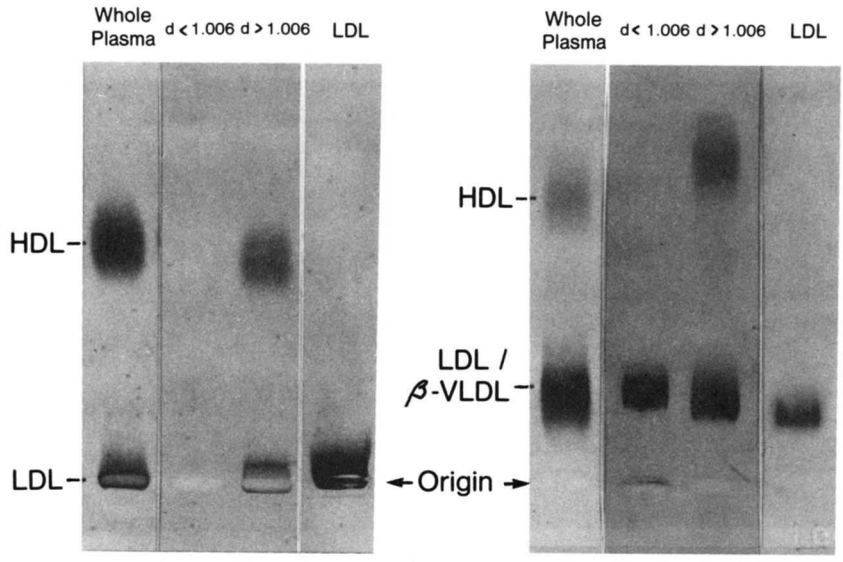

Fig. 5. Agarose electrophoresis patterns of whole plasma and isolated lipoprotein fractions. Typical examples of the electrophoretic pattern of whole plasma, d > 1.006 and d < 1.006 g/ml fractions and LDL isolated by 4% agarose column choromatography from grain-fed (left panel) and cholesterol-fed (right panel) Show Racer pigeons. The plasma cholesterol concentration of the hypercholesterolemic plasma used for these experiments was 1680 mg/dl.

tween 43 and 67 K as seen in Fig. 10. The apoprotein patterns for LDL from cholesterol-fed birds were virtually identical over a range of molecular weight of 4.1 to 11.7 $\times 10^6$. An apoprotein with a mobility of mammalian apoE was never seen. Again, no quantitative or qualitative differences between WC and SR pigeons were observed in the apolipoproteins.

HDL apoproteins are shown in **Fig. 12.** The major apoprotein of HDL from both grain-fed and cholesterol-fed birds was apoA-I. Two other proteins were also consistently observed in pigeon HDL. One had a molecular weight of slightly greater than 67 K and the other migrated between the 43-K and 67-K standards. Little difference was seen between the apoprotein patterns of WC or SR pigeons, males or females. Unlike the other lipoproteins, there were no major qualitative differences in apoproteins of HDL from grain- or cholesterol-fed birds.

DISCUSSION

White Carneau pigeons are one of the few animal models that develop severe atherosclerosis spontaneously while consuming a cholesterol-free diet. Show Racer pigeons of the same age are resistant to development of atherosclerosis even though both breeds have virtually the same plasma cholesterol concentrations (3). Upon cholesterol feeding there is a marked increase in total plasma cholesterol concentrations in both breeds and an acceleration in the rate of development of atherosclerosis, yet WC

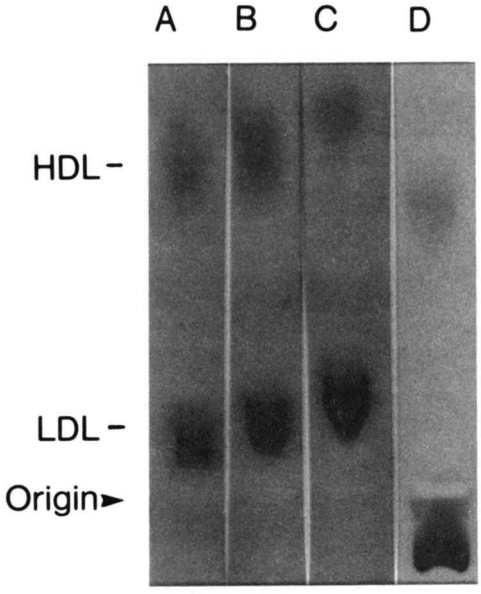

Fig. 6. Agarose electrophoresis patterns of whole plasma from male Show Racer pigeons with different degrees of hypercholesterolemia. Plasma from cholesterol-fed pigeons with different degrees of hypercholesterolemia is shown in A–C. Also included is whole plasma from an egg-laying female with typical gross hypertriglyceridemia (D). All separations were run at the same time on the same gel with 20 μl of plasma applied per lane.

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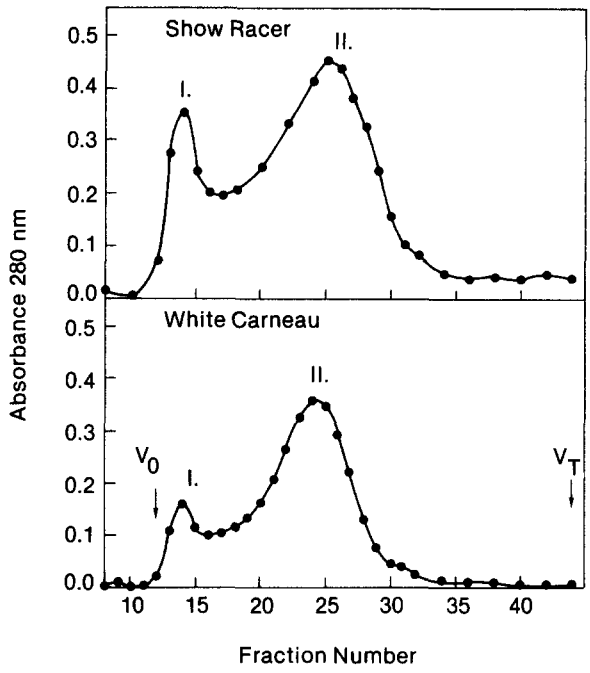

Fig. 7. Typical elution pattern of d < 1.006 g/ml lipoproteins isolated from cholesterol-fed animals and separated by chromatography on 2% agarose (Bio-Gel A-50m).

pigeons retain their greater susceptibility to atherosclerosis relative to SR (3). The explanation for this difference is unknown, but one possibility is that there are differences in the composition and/or concentration of specific lipoproteins of one breed that mediate the differences in atherosclerosis susceptibility. The principal purpose of this study was to characterize the lipoproteins of WC and SR pigeons to determine whether there were quantitative or qualitative differences in the plasma lipoproteins that might support the above hypothesis.

Grain-fed pigeons are hypercholesterolemic relative to most animal species, having total plasma cholesterol concentrations of approximately 300 mg/dl. Consistent with earlier reports (18, 19, 30), 75–80% of the cholesterol was present as HDL, the remainder as LDL with only trace amounts of VLDL. Grain-fed pigeons have one of the highest HDL cholesterol concentrations of any species examined (17), averaging nearly 200 mg/dl. Under the same conditions LDL cholesterol concentrations average about 50 mg/dl and thus are about 30% of that seen in human beings. Nevertheless, this LDL concentration is higher than that found in most other animals consuming cholesterol-free diets (17, 31). The chemical composition, apoprotein composition, and electrophoretic mobility of pigeon HDL were similar to other species, including man (31), with the exception that apoA-I was the principal apolipoprotein and little if any apoA-II was detected (20). Only a single HDL subclass, similar to HDL_2, was present. This is consistent with results from chickens (32, 33) and with the absence of apoA-II. The lipid composition of LDL was also similar to the LDL of man (3) with the exception of somewhat higher triglyceride concentrations. A large molecular weight protein similar to apoB-100 was the major protein. There were also larger amounts of apoA-I and the presence of several other unidentified proteins not generally found in mammalian

TABLE 7. Percent composition of high density lipoproteins of grain- and cholesterol-fed pigeons at 5 months[a]

	Protein	Phospholipid	Free Chol.	Chol. Esters	Triglyceride
Grain-fed					
White Carneau					
All animals	41.1 (12)[b]	36.1	3.7	15.5	3.5
Males	45.3 (5)	33.4	5.6	14.2	1.4
Females	38.1 (7)	38.1	2.4	16.4	3.8
Show Racer					
All animals	42.8 (12)	27.1	6.2	12.8	10.9
Males	42.3 (4)	32.8	5.5	15.7	3.6
Females	43.0 (8)	24.2	6.6	11.3	14.6
Cholesterol-fed					
White Carneau					
All animals	44.9 ± 1.6 (17)	31.1 ± 1.4	3.9 ± 0.3	17.2 ± 1.7	2.9 ± 0.6
Males	46.8 ± 2.5 (8)	28.1 ± 1.5	4.2 ± 0.4	18.8 ± 1.2	2.1 ± 0.6
Females	43.2 ± 2.1 (9)	33.7 ± 1.9	3.6 ± 0.4	15.8 ± 1.8	3.7 ± 1.0
Show Racer					
All animals	46.8 ± 1.3 (16)	29.8 ± 1.0	3.6 ± 0.3	18.9 ± 0.7	1.9 ± 0.3
Males	49.8 ± 1.9 (5)	27.1 ± 1.4	3.5 ± 0.6	17.9 ± 2.0	1.8 ± 0.3
Females	45.4 ± 1.5 (11)	31.0 ± 1.1	3.7 ± 0.3	19.1 ± 0.7	1.9 ± 0.4

[a]Results are the mean ± SEM for the cholesterol-fed animals and the mean of duplicate determinations of the HDL obtained from the pooled plasma from grain-fed birds as described in the legend to Table 4.
[b]Values in parentheses represent the number of animals per group and apply for all parameters indicated.

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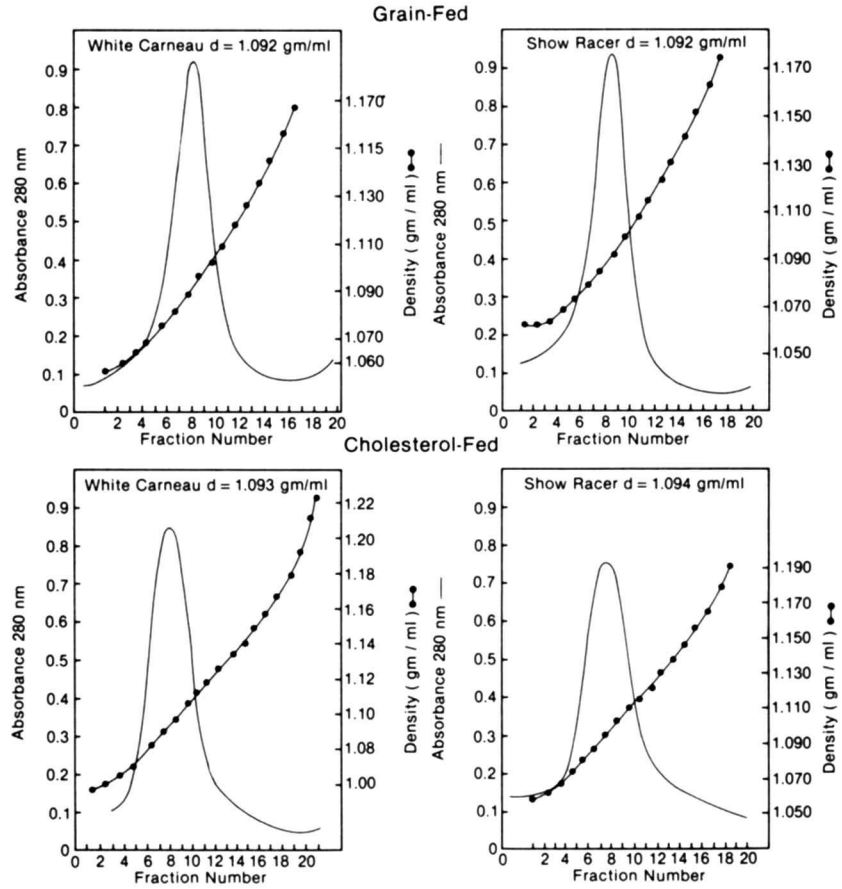

Fig. 8. Separation of pigeon HDL by density gradient ultracentrifugation.

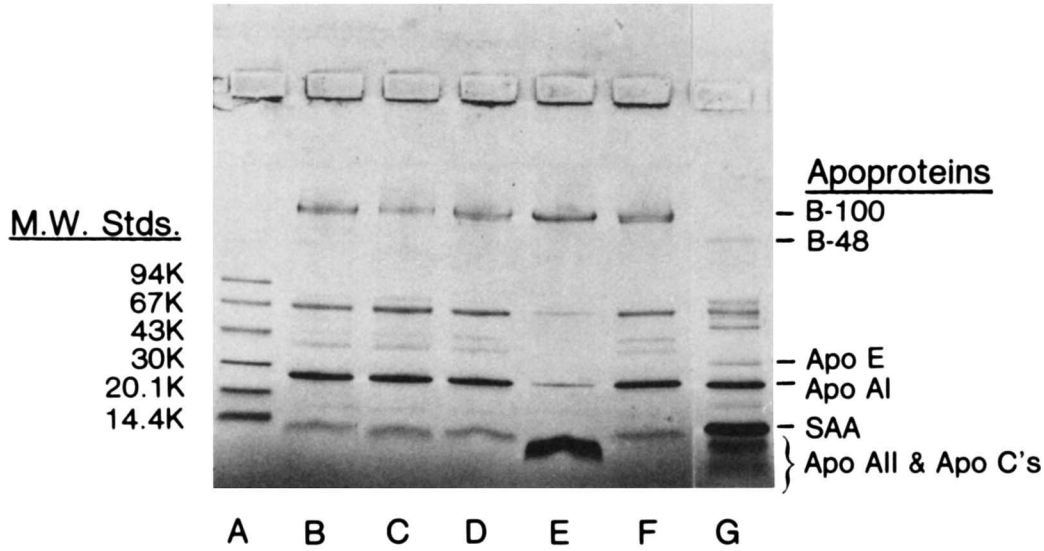

Fig. 9. SDS gradient gel (4–30%) electrophoresis of d < 1.006 g/ml (β-VLDL) apoproteins from cholesterol-fed pigeons. Forty μg of protein was placed on each lane of the gel. In lane A is the molecular weight standard (Pharmacia LMW stds) consisting of phosphorylase b, 94 K; albumin, 67 K; ovalbumin, 43 K; carbonic anhydrase, 30 K; trypsin inhibitor, 20.1 K; and α-lactalbumin, 14.4 K. The identity of the lipoproteins in the other lanes is as follows; B and C, SR female; D, SR male; E, WC female in egg-laying phase; F, WC male; G, African green monkey lymph chylomicrons. All lipoproteins were from the 5-month period.

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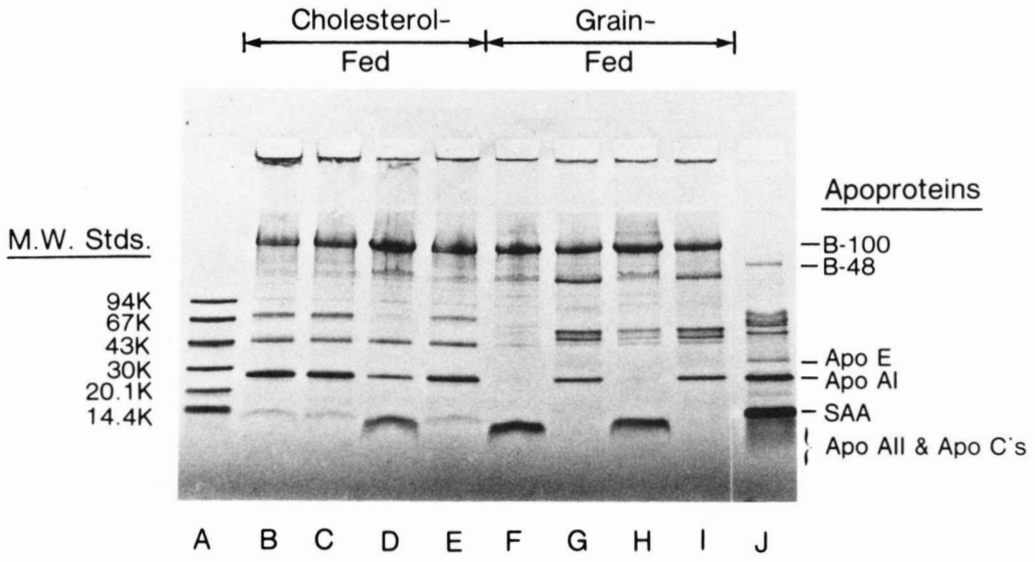

Fig. 10. SDS gradient gel (4–30%) electrophoresis of LDL apoproteins from grain- and cholesterol-fed pigeons. The amount of protein applied per sample and the identity of the molecular weight standards (lane A) are described in the legend of Fig. 9. The identity of the lipoproteins in the other lanes is as follows: B, SR female; C, SR male; D, WC female in egg-laying phase; E, WC male; F, SR female in egg-laying phase; G, SR male; H, WC female in egg-laying phase; I, WC male; J, rhesus monkey lymph chylomicrons. All lipoproteins were from the 5-month period.

LDL. Whether these apoproteins are on the same particle as the apoB or whether they are on separate particles is not known. The presence of these proteins probably does not result from contamination with other plasma proteins since the LDL was purified by agarose column chromatography prior to apoprotein separation. The predominance of apoB in pigeon LDL is similar to that reported for the cockerel (31). A major difference between LDL from grain-fed pigeons and mammalian LDL was its electrophoretic mobility. Pigeon LDL barely migrated from the point of application, suggesting that its surface had less net negative charge than mammalian LDL. Simi-

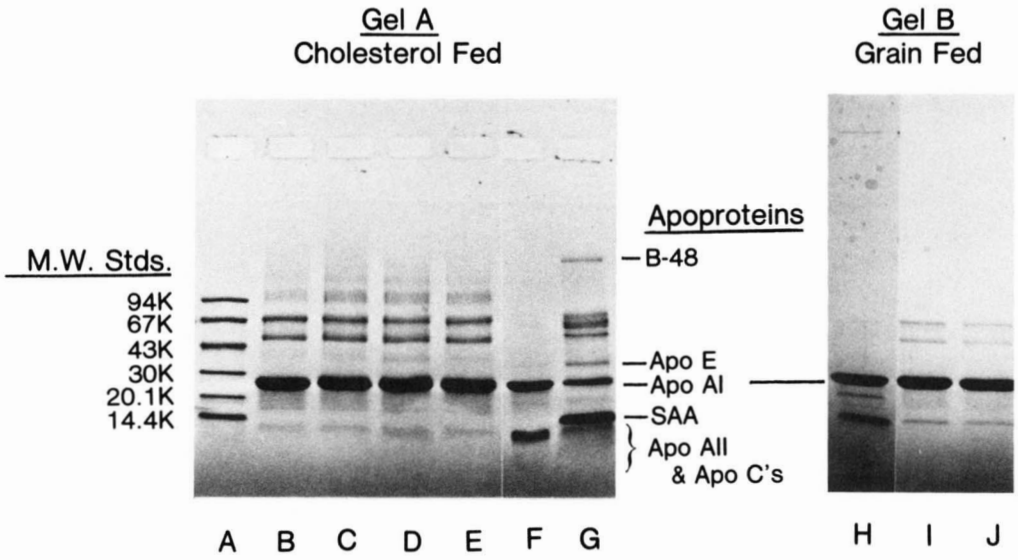

Fig. 11. SDS gradient gel (4–30%) electrophoresis of LDL apoproteins from LDLs of different molecular weight. The amount of protein applied per sample and the identity of the molecular weight standards (lane A) are described in the legend of Fig. 9. All LDL are from male cholesterol-fed animals except for lanes F and K from grain-fed animals. The identity of the lipoproteins in the other lanes and their molecular weights ($\times 10^{-6}$) are as follows: B, SR 11.7; C, SR 9.7; D, SR 7.5; E, SR 5.1; F, SR 3.2 (grain-fed); G, WC 8.4; H, WC 7.1; I, WC 6.1; J, WC 4.1; K, WC 3.2 (grain-fed); L, rhesus monkey lymph chylomicrons. All lipoproteins were from the 5-month period.

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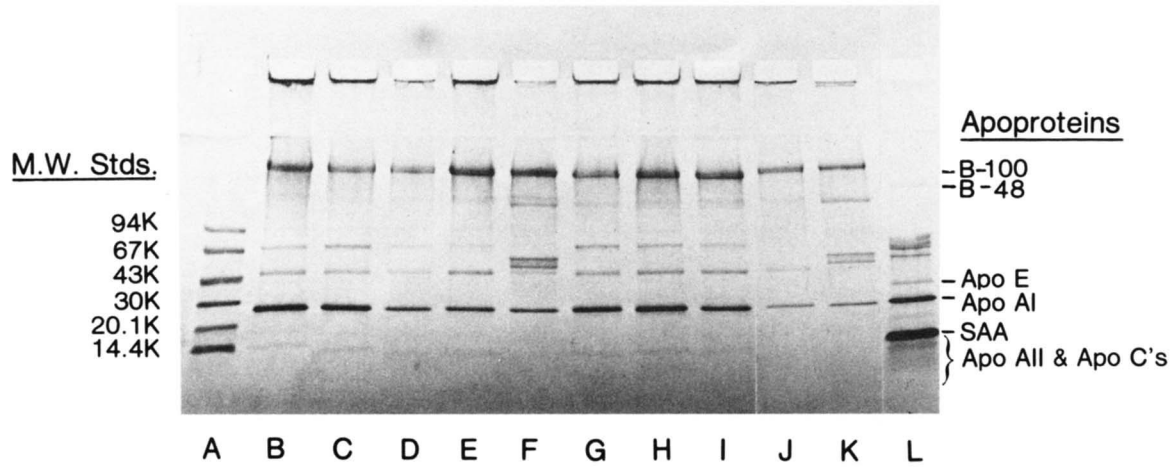

Fig. 12. SDS gradient gel (4–30%) electrophoresis of HDL apoproteins from grain- and cholesterol-fed pigeons. The amount of protein applied per sample and the identity of the molecular weight standards (lane A) are described in the legend of Fig. 9. Gel A and gel B were run separately. Lipoproteins from cholesterol-fed animals were from the 5-month period and for the grain-fed animals were from the 2-month period. The identity of the lipoproteins in the other lanes is as follows: B, SR female; C, SR male; D, WC female; E, WC male; F, rhesus monkey HDL; G, rhesus monkey lymph chylomicrons; H, rhesus monkey HDL; I, WC male; J, SR male.

lar results were reported by Langelier, Connelly, and Subbiah (18) using paper electrophoresis. There were no consistent differences in any of the above described lipoprotein parameters between grain-fed WC and SR pigeons.

Cholesterol feeding caused marked changes in the composition and content of the plasma lipoproteins of both breeds of pigeons. At plasma cholesterol concentrations less than about 600–700 mg/dl, LDL and HDL were the principal lipoproteins. Above 600–700 mg/dl, a beta-migrating VLDL was consistently present as a major lipoprotein fraction, with many properties similar to β-VLDL from several cholesterol-fed mammalian species including dog, rabbit, rat, swine, and monkey (for review see ref. 34). β-VLDL increased in concentration as plasma cholesterol concentrations increased. In some animals with plasma cholesterol concentrations greater than 2000 mg/dl, β-VLDL accounted for greater than one-half of the plasma cholesterol. LDL concentrations also increased greater than 10-fold in cholesterol-fed animals. HDL, however, showed little change in either content, composition, or density with cholesterol feeding. This is in contrast to several nonhuman primate species (34–36) and rabbits (37) in which HDL concentrations decrease with hypercholesterolemia.

The β-VLDL of hypercholesterolemic pigeons was cholesteryl ester-rich, floated at a density less than 1.006 g/ml, and had β-mobility on electrophoresis. ApoB-100, a protein of approximately 67 K, and apoA-I were the major apoproteins. Unlike β-VLDL from dogs and rabbits (13, 38), pigeon β-VLDL had no detectable apoE or apoB-48. There was considerable variation in the concentration of β-VLDL among individual animals, as there was in the amount of atherosclerosis. The extent to which the individual plasma lipoproteins correlate with extent of atherosclerosis will be the subject of a subsequent report.

In cholesterol-fed animals LDL and β-VLDL transported about equivalent amounts of cholesterol. In addition to an increased number of LDL particles there was a marked increase in the number of cholesteryl ester molecules per particle from approximately 1300 to over 10,000 for the most hypercholesterolemic LDL. This resulted in an increase in LDL particle size from $M_r = 3.2 \times 10^6$ for normal LDL to greater than $M_r = 10 \times 10^6$ for the largest LDL. The increase in LDL size with cholesterol feeding is similar to that seen with certain species of cholesterol-fed nonhuman primates (7, 10) except that the enlargement of the LDL was greater than that seen for nonhuman primates. ApoB-100 and apoA-I were the major apoproteins of LDL from cholesterol-fed animals. This apoprotein pattern was remarkably similar for LDL of cholesterol-fed animals regardless of the molecular weight.

In addition to the increased size of the LDL from hypercholesterolemic animals these LDL also had an altered electrophoretic mobility relative to LDL from grain-fed animals. Whereas the normal LDL migrated little from the point of application, the LDL from hypercholesterolemic animals migrated with β-mobility similar to human LDL. This change in electrophoretic mobility occurred progressively with increasing plasma cholesterol concentrations up to about 1500 mg/dl. As a result there must have been a gradual change in the surface charge of the LDL particle as it became larger. Whether this was due strictly to conformational changes of the apoB on the surface of the LDL or due to changes in some of the

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minor apoproteins found on LDL from cholesterol-fed animals (Fig. 10) is unknown. Even though cholesterol feeding alters the electrophoretic mobility of pigeon LDL, LDL from both grain-fed (39) and cholesterol-fed (unpublished data) pigeons have the capacity to bind to mammalian LDL receptors.

Not only was there a change in electrophoretic mobility of LDL with hypercholesterolemia, there was also a similar change in electrophoretic mobility of β-VLDL. This suggests the possibility that LDL and β-VLDL are not discrete particles but rather a continuum of particles that range from density < 1.006 g/ml to 1.063 g/ml. Two pieces of evidence argue against this conclusion. First, the apoproteins of β-VLDL differ from those of LDL by lacking a 47-K protein that is present in LDL. Second, separation of the total plasma lipoproteins by agarose gel chromatography indicates the presence of discrete LDL and β-VLDL peaks (3).

The presence of two subclasses of β-VLDL is similar to what has been reported for β-VLDL from cholesterol-fed dogs and from humans with type III dyslipoproteinemia (38). Peak I β-VLDL (the fraction eluting near the V_0) from dogs and humans appears to be of intestinal origin based on the presence of apoB-48 and the marked decrease in the peak I material after a 48-hr fast. In two experiments with WC pigeon β-VLDL (separated as shown in Fig. 7), the peak I β-VLDL apoprotein pattern was indistinguishable from that shown in Fig. 9 for total β-VLDL (data not shown). Neither apoB-48 nor apoE were present. Whether this implies that the peak I β-VLDL from pigeons is not of intestinal origin is unclear since pigeons, like all birds, do not absorb dietary lipid via lymphatic chylomicrons but rather directly into the portal system (40) by what has been termed portamicrons (31). This results in the formation of triglyceride-rich VLDL (40). Whether cholesterol and triglycerides are absorbed as part of the same portamicron particle has not been examined. The process of intestinal absorption of lipids presumably requires active protein synthesis by the intestine. This process does not appear to involve the production of apoB-48 as occurs in mammals, unless, of course, B-48 is efficiently removed by the liver during the initial passage of portamicrons through the liver and thus does not accumulate in the peripheral blood.

Major changes are known to occur in the plasma lipoproteins of birds during their egg-laying phase (for review see ref. 31) that can be mimicked by estrogen administration (32). These changes include a marked hypertriglyceridemia, hypercholesterolemia, an increase in the VLDL and LDL fractions (as determined by flotation at standard density cuts), and a decrease in HDL. The major lipoprotein in the egg-laying hen (41) or estrogen-treated cockerel (42) responsible for the transport of these massive amounts of lipid has been termed VLDL-II. Evidence suggests that there may only be a single lipoprotein, VLDL-II, in estrogen-treated chickens, but that it has a broad density range such that it can be isolated in both the LDL and VLDL density ranges (32). VLDL-II contains two major proteins, apoB (approximately 35%) and apoVLDL-II (approximately 65%). In immature hens and cockerels only apoB is present. ApoVLDL-II is a dimeric protein with a molecular weight reported to be either 14 K (32) or 18 K (41). When separated by SDS gel electrophoresis under reducing conditions in the presence of mercaptoethanol, the disulfide linkage is resistant to reduction; thus, some of the dimeric form may be seen even under mild reducing conditions. VLDL-II functions to transport lipids and proteins across the oocyte into the developing egg. Chapman (31) has suggested that apoVLDL-II is the ligand responsible for binding to receptors on the oocyte membrane, while Perry, Griffin, and Gilbert (43) suggest that apoB may serve this function.

Female pigeons in the egg-laying phase have changes in their lipoproteins similar to those described for the chicken. Plasma triglyceride concentrations as high as 3800 mg/dl were observed in some female pigeons in this study. Upon electrophoresis of whole plasma there is a marked change in electrophoretic mobility, with the major lipoprotein remaining at the origin even in cholesterol-fed animals (Fig. 6). Both β-VLDL and LDL became triglyceride-rich. This occurred in both grain- and cholesterol-fed animals. In the presence of hypercholesterolemia, the hypertriglyceridemia of the egg-laying cycle was associated with the displacement of some of the cholesteryl esters of the β-VLDL and LDL with triglycerides. The total amount of core components remained relatively constant, however. The apoprotein changes in egg-laying pigeons also mimicked the changes reported in chickens. This is seen most clearly in Fig. 9E and Fig. 10D, F, and H in which a protein of approximately 10–12 K is seen to make up a substantial portion of the apoproteins of β-VLDL and LDL. Because our gradient gels were not designed to resolve proteins in this molecular weight range, we can only estimate the molecular weight of this apoprotein. It is, nevertheless, within the size range reported for chicken apoVLDL-II. The other major apoprotein is apoB. It is unclear what role the periodic appearance of VLDL-II might play in influencing atherosclerosis development in pigeons. Since no major sex differences in aortic atherosclerosis have been reported in WC pigeons (44), the influence of VLDL-II appears to be minor.

Although there were several differences and similarities of pigeon lipoproteins with mammalian lipoproteins, there were no systematic differences in either the composition or concentration of the lipoproteins of the two breeds of pigeons that would help to explain the susceptibility of the WC pigeon or the resistance of the SR pigeon to aortic

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atherosclerosis. This implies that factors that mediate atherosclerosis susceptibility in pigeons may instead reside at the level of the arterial wall. These might include differences in the rate of influx of the plasma lipoproteins or in the interaction of the cellular components of the atherosclerotic plaque, either monocyte-macrophages, endothelial cells, or smooth muscle cells, with the plasma lipoproteins.

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