Changes in the concentrations and distributions of apolipoproteins of the aging rat

Brian J. Van Lenten and Paul S. Roheim[1]

Department of Physiology, Louisiana State University Medical Center, New Orleans, LA 70119

Abstract The hyperlipidemia associated with aging was characterized in the rat by comparing the plasma lipid, lipoprotein, and apolipoprotein profiles of adult (12 weeks old) and old (96 weeks old) male rats. Compared with those of the adult rats, the VLDL concentrations of the old rats were reduced, but IDL, LDL, and HDL concentrations were elevated. Despite a reduced VLDL concentration, concentrations of triglycerides in the plasma of the old rats were elevated. This phenomenon was attributed to an enrichment of triglyceride in the other lipoprotein fractions. In the old rats, hypercholesterolemia was the result of elevated IDL- and HDL-cholesterol whereas elevated plasma concentrations of apolipoproteins B and E were attributed to elevated LDL and HDL concentrations, respectively. Although concentrations of apolipoproteins A-I and A-IV did not change significantly in the plasma of the old rats, the distribution pattern of the apoA-IV was altered dramatically. Compared with the adult rats, a shift of apoA-IV in the HDL to the "lipoprotein-free" fraction was observed in the old rats, as measured by agarose gel chromatography. The data demonstrate that the hyperlipidemia in the old rats is associated with selective changes in the apolipoprotein profile.—**Van Lenten, B. J., and P. S. Roheim.** Changes in the concentrations and distributions of apolipoproteins of the aging rat. *J. Lipid Res.* 1982. **23:** 1187–1195.

Supplementary key words apolipoprotein profiles • "lipoprotein-free" state of apolipoproteins • aging and lipoprotein metabolism

There is a close relationship among aging, atherosclerosis, and hyperlipidemia (1, 2). In the rat, a widely used animal model in lipid and aging research, plasma and tissue lipids have been found to increase with age (3–6). Uchida et al. (7) found that lipids in serum very low density lipoproteins decreased with age in rats, whereas lipids in low density lipoproteins and high density lipoproteins increased. The LDL and HDL of old rats appear to be especially cholesterol-enriched (7, 8).

The apolipoproteins not only are important structural components of lipoproteins, but also function in lipoprotein metabolism as cofactors for enzymatic reactions and in receptor-mediated uptake of lipoproteins (9, 10). Alterations of apolipoprotein profiles and/or changes in receptors could result in hyperlipidemia. Therefore, possible changes in apolipoprotein concentrations and/or distribution may contribute to the observed hyperlipidemia associated with aging. As a first step in attempting to answer this question, we compared adult and old rats to determine whether aging results in alterations in apolipoprotein profiles.

MATERIALS AND METHODS

Animals

Male Wistar rats born and raised in our colony and aged 12 weeks (designated "adult") and breeder rats aged 96 weeks (designated "old") were permitted water and Purina Mouse Breeder Lab Chow (Ralston Purina Co., St. Louis, MO) ad libitum. They were weighed weekly after weaning to assess their growth rates and health. Compared with a regular low-fat meal (3% fat), Breeder's chow (11% fat) produces a moderate elevation of fasting plasma lipid values in rats. Between 1300 and 1500 hours, the animals were bled from the abdominal aorta under light anesthesia and killed. After separation from the cells by low-speed centrifugation, sera were preserved with a solution of 0.01% EDTA and 0.01% sodium azide.

Lipoprotein isolation

Ultracentrifugation. Lipoproteins were isolated by ultracentrifugation according to the technique of Havel, Eder, and Bragdon (11). Ultracentrifugation was carried out in a Beckman L5-50 ultracentrifuge using a 40.3 rotor with adaptors that permit ultracentrifugation of 1-ml samples. The VLDL ($d < 1.006$ g/ml), IDL (d 1.006–1.030 g/ml), LDL (d 1.030–1.063 g/ml), HDL (d 1.063–1.210 g/ml), and the $d > 1.210$ g/ml

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; "lipoprotein-free" fraction, $d > 1.210$ g/ml; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; QEIA, quantitative electroimmunoassay; A.U., arbitrary units.

[1] Address all correspondence to Dr. Paul S. Roheim, Department of Physiology, LSU Medical Center, 1542 Tulane Avenue, New Orleans, LA 70112.

or "lipoprotein-free" fraction of the serum were studied. For ultracentrifugation involving VLDL, IDL, and LDL, a single 24-hr spin at 100,000 *g* was used. A single 48-hr spin at 100,000 *g* was used to isolate HDL. All centrifugations were carried out at 15°C. The pellet in the bottom of the ultracentrifuge tube was disrupted and mixed thoroughly.

Agarose gel chromatography. Gel chromatography was also used to separate the lipoproteins. A 0.9 × 90 cm column of 6% agarose (Bio-Gel A-0.5M 200–400 and A-5M mesh, Bio-Rad Laboratories, Richmond, CA) were used with a buffer of 0.15 M NaCl, 0.01% EDTA, and 0.01% sodium azide, pH 7.4. Whole serum samples of 2 ml were applied to the column immediately after separation from the cells. The column was operated at 23°C and a flow rate of 5.3 ml/hr was maintained throughout the column runs. Elution fractions of 1.1 ml were collected. The column was calibrated with blue dextran, which showed the void volume to be 27.5 ml. In addition, human ^{125}I-labeled LDL and ^{125}I-labeled albumin were used as internal standards for each column run. The radiolabeled human albumin marks the region of the elution profile containing the smaller plasma proteins, where the free apolipoproteins would elute. In this way, an indication of what is conventionally called the "lipoprotein-free" fraction could be made and the data compared with results obtained from ultracentrifugal fractions.

Apolipoprotein quantitation. Apolipoproteins were measured by quantitative immunoelectrophoresis according to the technique of Laurell (12) and modified by Bar-On, Roheim, and Eder (13). In addition, a nonionic detergent, Nonidet-40 (NP-40), was added to a final concentration of 1% in the samples and standards and 0.05% in the agarose to measure concentrations of apolipoproteins A-I and E (14). To reduce the loss of apolipoproteins as a result of repeated ultracentrifugations, we estimated the apolipoprotein content of the different lipoprotein fractions by subtracting the peak heights of successive infranatant fractions after a single-spin ultracentrifugation. Thus, the difference between the peak heights of whole serum and the d > 1.006 g/ml fraction (infranate) represents d < 1.006 g/ml (VLDL). This method also permits the determination of possible losses of apolipoproteins due to this procedure. The albumin concentration in each of the d > (infranatant) fractions should be the same as the albumin concentration in the whole plasma, because no albumin should be removed in any of the d < (supernatant) fractions. By determining actual albumin concentrations in plasma and in the d > fractions, we can correct for nonspecific protein losses. (See legend to Fig. 2.)

The antisera used in this study were prepared as previously described (13). Apolipoprotein values from the determinations, expressed as arbitrary units (A.U.), were calculated as percentages of a rat plasma standard pool run simultaneously on the plates with the samples and a secondary standard. All experimental plasma samples as well as rat standard pools were stored at −20°C. Using QEIA one should be aware of the specific requirements necessary for quantitation as stated by Laurell (12). That is, the validity of the method requires that the antigen occur with identical molecular size and charge in the sample and standards. Standards of purified proteins do not always satisfy this criterion. To eliminate methodologic problems arising from the use of standards in which the protein is in a different state than in normal serum, we expressed all data in arbitrary units.

SDS gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (15) was used to evaluate the apolipoprotein composition of the d < 1.210 g/ml fraction. Acrylamide gels of 10% and 3.5% were used to separate the major apolipoproteins and the two major forms of apoB, respectively. The protein bands were stained with 0.1% Coomassie Blue G-250, and scanned by a E-C Apparatus Corp. Densitometer.

Chemical quantitation and statistical analysis

Plasma triglyceride and cholesterol concentrations were determined according to the methods of Bucolo and David (16) and Allain et al. (17), respectively. Protein was determined according to the method of Lowry et al. (18), using bovine serum albumin as the standard. Student's "*t*" distribution was used to statistically analyze the data, and a *P* value of < 0.05 was considered significant.

RESULTS

Table 1 presents the body weights and the plasma levels of the major lipids and apolipoproteins of adult and old rats. The old rats were about 75% heavier than the adult rats. Both plasma cholesterol and triglyceride concentrations were also significantly increased with age, as were the plasma apolipoproteins, apoB and apoE. Neither apoA-I nor apoA-IV concentrations significantly changed in the plasma.

To determine the distribution of the lipoprotein components among the lipoprotein fractions, we measured apolipoprotein and lipid contents in ultracentrifugally isolated fractions. Old rats had significantly more cholesterol in IDL and HDL than did adult rats (**Fig. 1**). Although VLDL-triglyceride was greatly reduced in the

old rats from 126.5 mg/dl to 8.3 mg/dl, triglyceride concentrations increased in the d > 1.006 g/ml fraction from 24.5 mg/dl to 242.7 mg/dl in the old rat. These changes are consistent with the changes in total protein of the lipoprotein fractions, i.e., VLDL-protein decreased (8.1 mg/dl to 5.7 mg/dl) whereas IDL-, LDL-, and HDL-protein increased with age (0.2 mg/dl to 0.7 mg/dl; 5.9 mg/dl to 9.1 mg/dl; and 54.9 mg/dl to 65.6 mg/dl, respectively).

Figs. 2A–D show the ultracentrifugal distribution of the apolipoproteins of old and adult rats. In the old rats, VLDL-apoB was reduced, whereas apoB was elevated in LDL and HDL (Fig. 2A), thus accounting for the increased total apoB in the serum of old rats. Fig. 2B shows that the overall increase of apoE in the serum of old rats is due to significant increases in HDL-apoE and in apoE of the d > 1.210 g/ml fraction. Insofar as the total plasma apoA-I concentration did not change statistically with age, the accumulation of apoA-I in the d > 1.210 g/ml fraction was greater in the old rat (Fig. 2C). The most striking alteration in apolipoprotein distribution, however, occurred with apoA-IV. Although the old rats had the same plasma apoA-IV concentration as adult rats, the apoA-IV concentration decreased considerably in the lipoprotein fractions while dramatically accumulating in the d > 1.210 g/ml fraction.

Column chromatography

We compared the data obtained by ultracentrifugation with data collected in the absence of ultracentrifugal artifacts (19) by fractionating fresh rat sera by agarose gel chromatography. Because the most striking changes were observed in the HDL and the d > 1.210 g/ml fractions using ultracentrifugation, A-0.5M agarose was used to evaluate these two fractions by separating the lipoproteins from the smaller plasma proteins. Similar to the ultracentrifugation data, apoB concentrations were greater in old rats throughout the elution profiles for this apolipoprotein (**Fig. 3A**). The void volume contained the peak of the plasma apoB concentration, as determined by QEIA, which corresponded to the same tube containing the peak radioactivity of the ^{125}I-labeled LDL standard (Tube #25). The radioactive albumin consistently peaked at Tube #40.

TABLE 1. Plasma concentrations of lipids and apolipoproteins and the body weights of adult (12 weeks old) and old (96 weeks old) male rats[a]

	Adult	Old
Body weight, g	426 ± 16	740 ± 48[c]
Cholesterol, mg/dl	82 ± 3	165 ± 18[c]
Triglyceride, mg/dl	151 ± 4	251 ± 18[c]
ApoB, A.U.[b]	112 ± 5	154 ± 6[c]
ApoE, A.U.	87 ± 4	165 ± 3[c]
ApoA-I, A.U.	150 ± 5	175 ± 16
ApoA-IV, A.U.	85 ± 2	83 ± 3

[a] Values are expressed as the mean ± SEM and are based on six animals per age group.
[b] A.U., arbitrary units.
[c] $P < 0.05$.

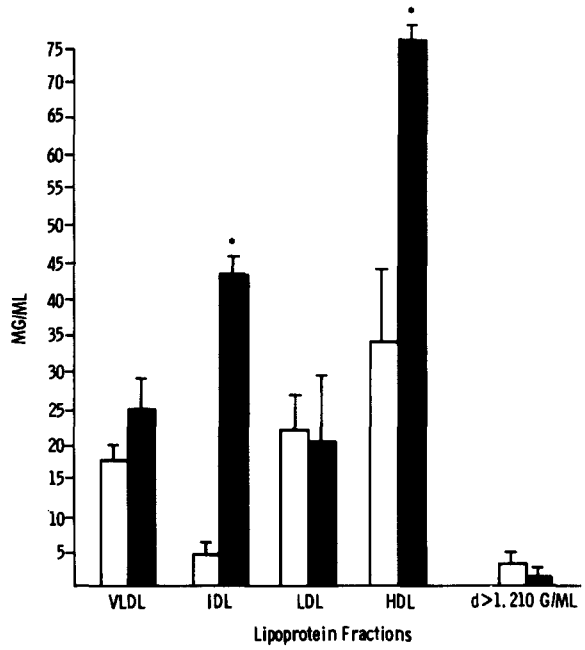

Fig. 1. Distribution of plasma cholesterol among the lipoprotein fractions of adult (□) and old (■) male rats. Values are based on three pools of two rats per pool. *, $P < 0.05$.

Separation of lipoproteins by A-0.5M chromatography showed that the data obtained using ultracentrifugation may have indicated an overestimation of the amount of apoE and apoA-I in the d > 1.210 g/ml fraction of old rat plasma (Figs. 3B and C). By ultracentrifugation, about 40% of the total apoE and 10% of the total apoA-I were found in the d > 1.210 g/ml fraction in the old rats, whereas by agarose gel chromatography, only trace amounts of apoE and apoA-I in young or old rats was found in "lipoprotein-free" fractions of both groups. Old rats, however, appeared to have had more apoE across the apolipoprotein elution spectrum. The peak of the apoA-I elution profile appeared to be shifted slightly to the right, towards a smaller particle size, in older rats. The dramatic shift in apoA-IV distribution by column chromatography (Fig. 3D) corroborated the ultracentrifugation profile for this apolipoprotein. In old rats, the two distinct major peaks of the apoA-IV elution pattern were both shifted to the right, toward smaller particle sizes. Although adult rats had demonstrable amounts of apoA-IV in the peak corresponding to proteins smaller than albumin, old rats had almost half of their total apoA-IV in this area of the elution profile.

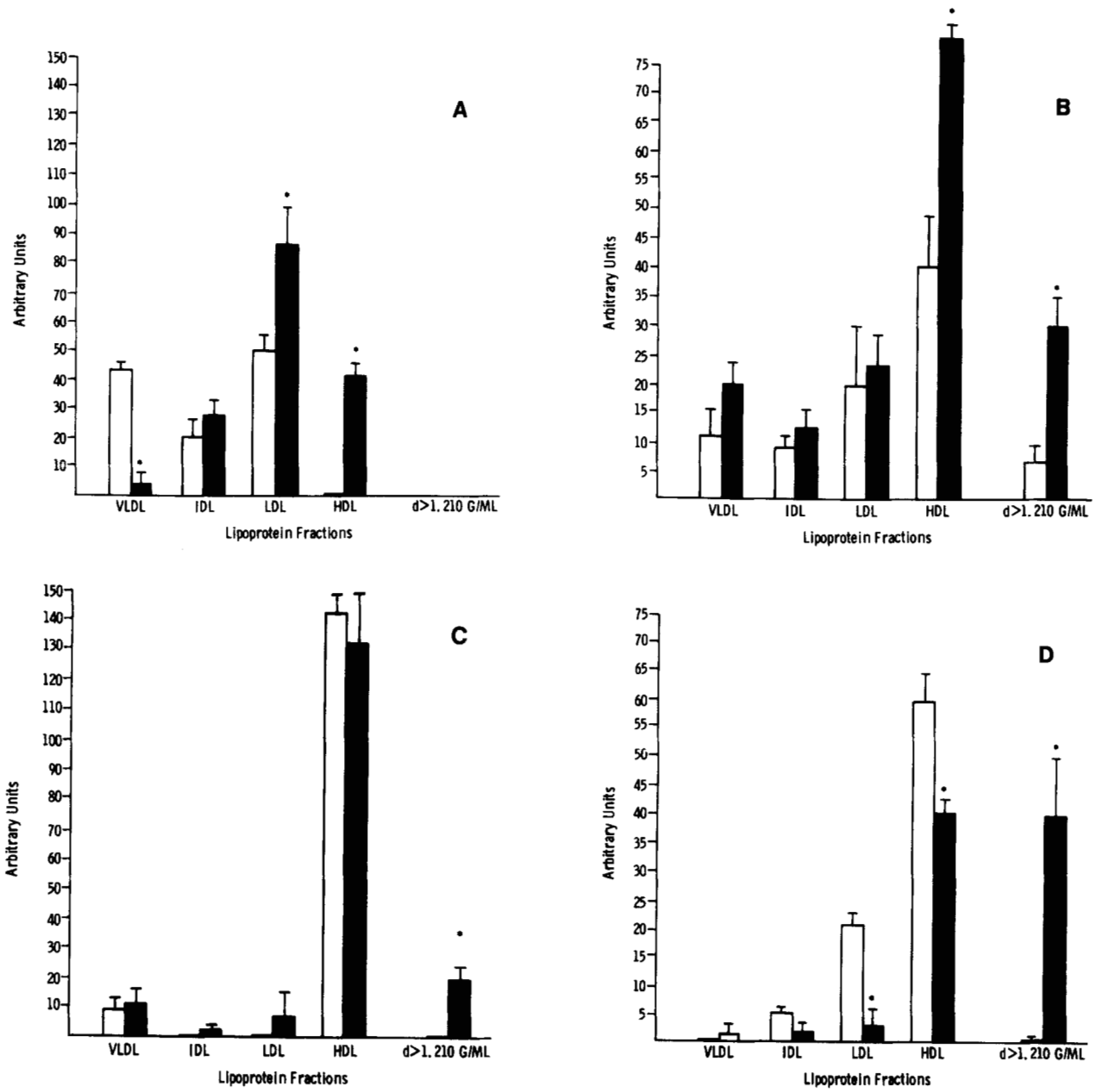

Fig. 2. Distribution of plasma apoB (A) and apoE (B), apoA-I (C), and apoA-IV (D) among the lipoprotein fractions of adult (□) and old (■) male rats. In order to compensate for possible losses of apoproteins due to isolation procedures, an albumin correction factor was designed. Since all the albumin should be recovered in the d > infranate fraction, the obtained apoprotein value of the infranate was multiplied by the plasma albumin/infranate albumin ratio to correct for any mechanical losses. This correction factor ranged ±5%. Values are based on three pools of two rats per pool. *, $P < 0.05$.

Results of A-5M agarose chromatography were consistent with the above observations, i.e., concentrations of apolipoproteins (**Fig. 4A**) B and E (Fig. 4B) were greater in old rats throughout their elution profiles, whereas apoA-I profiles (Fig. 4C) were not very different with age. The peak of the apoA-IV profile (Fig. 4D) in old rats was shifted to the right of that from younger rats; less of the apoA-IV was of large size and more was of smaller size.

SDS-PAGE

To characterize the lipoproteins of old and adult rats using a method independent of QEIA, we used SDS-PAGE. Compared with the adult rat, the old rat appeared to have a greater proportion of apoE (36% vs. 17%) but less apoA-IV (12% vs. 18%) and apoA-I (52% vs. 65%) in the d < 1.210 g/ml fraction of the plasma (**Fig. 5**). This finding is consistent with observations

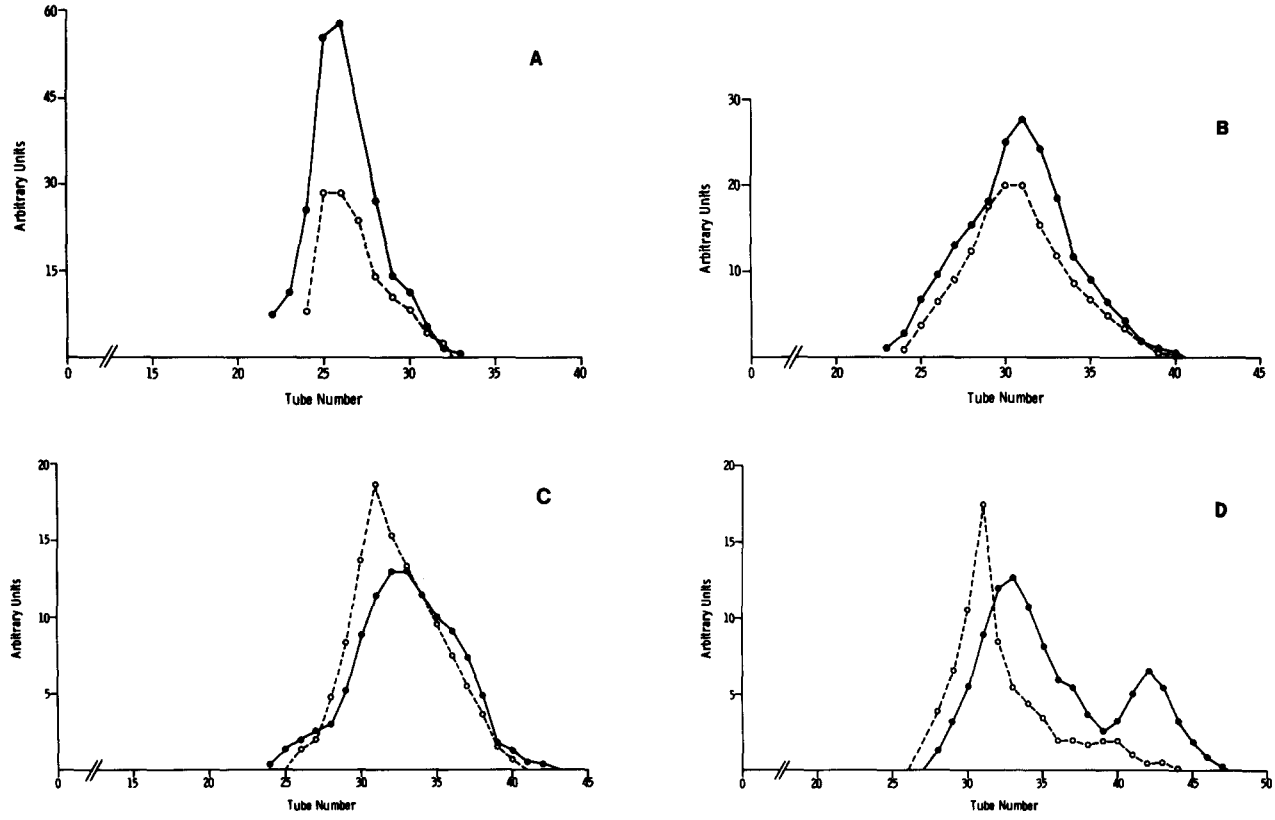

Fig. 3. A-0.5M agarose gel chromatography elution profiles for the apolipoproteins of adult (O – – – O) and old (● – – – ●) male rats. Two ml of freshly isolated serum from each rat was applied to the column and the concentrations of the apolipoproteins in each fraction were measured using the QEIA. (A) apoB. (B) apoE; (C) apoA-I; and (D) apoA-IV. Values are based on three rats per age group.

made using QEIA (see above). Because we found higher apoB concentrations in old rats, we further separated apoB by using 3.5% polyacrylamide gels to see whether the contents of high or low molecular weights forms of apoB differed in the old rat. We found, as shown in **Fig. 6,** that old rats, in addition to having more total apoB than adult rats, had a greater proportion of lower molecular weight apoB (L) than higher molecular weight apoB (H). In the adult rats the L/H ratio in the $d < 1.21$ fraction was 29%/71% or 0.41, whereas in old rats this ratio was twice as great—45%/55% or 0.82.

DISCUSSION

In this study we showed that the marked hyperlipidemia of old rats is associated with selective changes not only in the concentrations of the plasma lipoproteins, but also in the distributions of the lipids and the apolipoproteins within the lipoprotein classes. The hypertriglyceridemia of old rats is associated with a much different triglyceride profile than that found in adult rats. The triglyceride in VLDL is decreased with age, as found by others (7), whereas other fractions are all enriched with triglyceride. Hypercholesterolemia, on the other hand, was restricted to elevations in IDL- and HDL-cholesterol. In this study, however, we cannot conclusively eliminate the effect of an increased body weight on our observations. Nevertheless, in genetically obese Zucker rats, the changes observed in plasma lipoprotein profiles are very different from the changes in our study (20). In Zucker rats, hypertriglyceridemia appeared to be localized to VLDL whereas it is reduced in old rats. Total plasma apoB concentration is decreased while VLDL-apoB concentration increased in the Zucker rats (20). This should be compared with the present study in which total plasma apoB concentrations increased but VLDL-apoB was reduced in the old rats. Thus, this information suggests that changes observed in the obese Zucker rats are very different from changes found in the aged rats.

Apolipoprotein distribution was also altered with age in the rat. In spite of a reduced VLDL-apoB, old rats had an increased apoB concentration in the plasma, reflected by an increased LDL-apoB. Increased apoB levels were found in the HDL fraction using ultracentrifugation; however, A-5M agarose chromatography revealed no major difference in apoB distribution in the smaller (HDL) particles. Elevated HDL-apoE appeared to be primarily responsible for the increased plasma

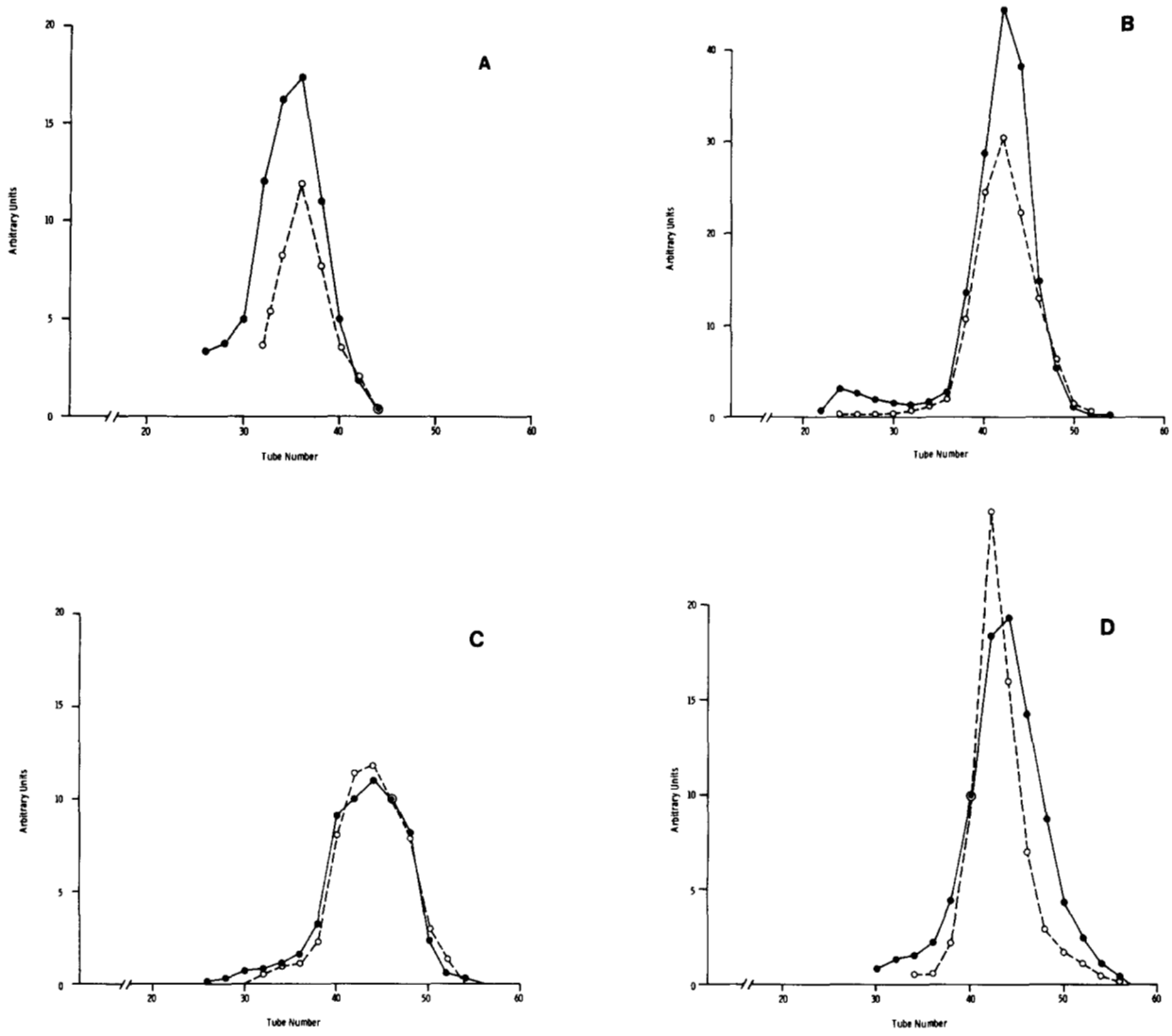

Fig. 4. A-5M agarose gel chromatography elution profiles for the apolipoproteins of adult (O – – – O) and old (● – – – ●) male rats. Two ml of freshly isolated serum for each rat was applied to the column, and the concentrations of the apolipoproteins in each fraction were measured using the QEIA. (A) apoB. (VLDL apoB values are not included); (B) apoE; (C) apoA-I; and (D) apoA-IV. Values are based on three rats per age group.

apoE concentration of old rats. These two apolipoproteins are known to have specific receptors on tissue membranes (10). Reductions in many nonlipoprotein receptor concentrations and/or activity have been found to be related to advancing age (21). Hui, Innerarity, and Mahley (22) recently showed that the liver membranes of young beagles had an increased binding activity for ^{125}I-labeled apo-E HDL_c compared with those of adult beagles, suggesting that lipoprotein receptors may also be affected by an animal's age or metabolic rate. One can envision a reduced lipoprotein receptor response resulting in a decreased uptake and utilization, whereby hyperlipidemia develops. This process may explain the elevated plasma LDL-apoB and HDL-apoE observed in the old rat.

Sparks and Marsh (23) showed that two major forms of apoB (a high and a low molecular weight species) appear to be metabolically different. According to these authors the lower molecular weight form is cleared more rapidly than the higher molecular weight form. Whether or not the observed enrichment of the LDL with the lower molecular weight form of apoB in the old rat is correlated with the hyperlipidemia of aging is not clear. It should be pointed out that the chromogenicity of the two forms of apoB was not determined. However, we assumed that the chromogenicities of the individual subspecies between the two groups were similar. We applied equal amounts of apoprotein to the gel, therefore the observed differences between adult and old rats should be a true reflection of their

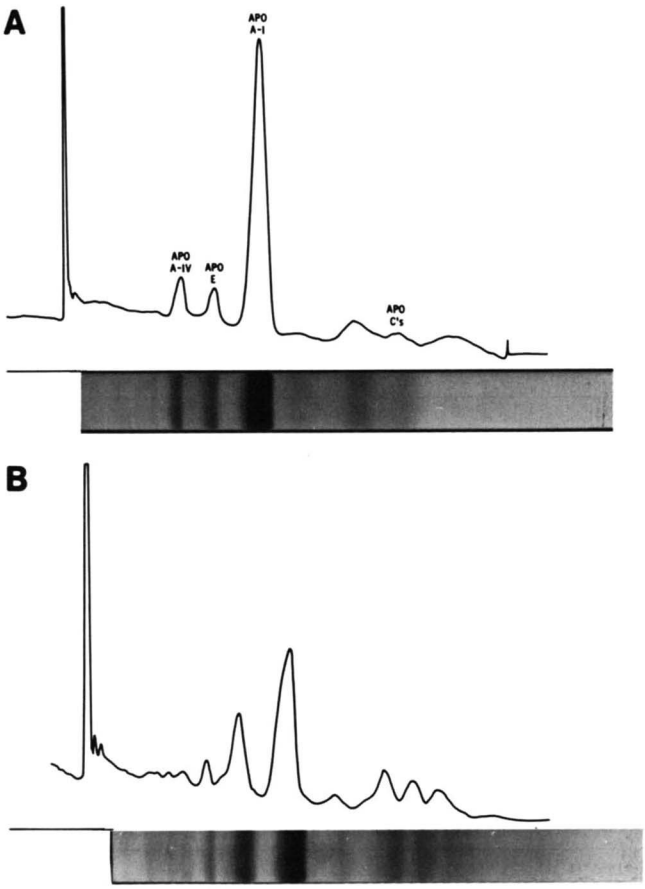

Fig. 5. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis utilizing gels of 10% acrylamide and densitometric scanning of the d < 1.21 g/ml fraction of adult (A) and old (B) male rats; 75 μg of apolipoprotein was applied to each gel.

apoB concentrations. It would be important to know whether this altered apoB ratio is due to a different synthesis of the B apolipoproteins and its subspecies in the old rat and/or is the result of different catabolism.

Old rats were shown to have less apoA-IV in the HDL fraction and more in the d > 1.210 g/ml fraction. This shift in distribution was also observed with agarose chromatography, however it was not apparent with apoE and apoA-I, confirming the finding of Fainaru, Havel, and Imaizumi (19). Therefore the presence of a large amount of apoA-IV in the "lipoprotein-free" fraction cannot be an ultracentrifugal artifact, as evidenced by the chromatographic elution of "free" apoA-IV after the albumin peak. It appears that this fraction of the plasma can be influenced by the hyperlipidemic state. Indeed, we have observed in our laboratory that a high-fat/high-cholesterol diet results in a shift of apoA-IV from HDL to the d > 1.210 g/ml fraction.[2] Interestingly, the high concentration of apoA-IV in the "lipoprotein-free" fraction of old rats is similar to the human apoA-IV profile (24, 25). The extent of the lipemia may possibly be directly related to the amount of apoA-IV in the d > 1.210 g/ml or the "lipoprotein-free" fraction.

We have demonstrated that "free apolipoproteins" exist in rat plasma, and these free apolipoproteins could combine with lipid to form lipoproteins (26). It has also been shown that apoA-IV, once released from triglyceride-rich lipoproteins during lipolysis, enters the d > 1.210 g/ml fraction of rat plasma (27). It then appears to become associated with the HDL fraction. ApoA-IV in the "free" state may associate with the HDL fraction after combining with lipid in the plasma or at the cell surface and then becoming less dense, or by directly binding to the HDL particle. The presence

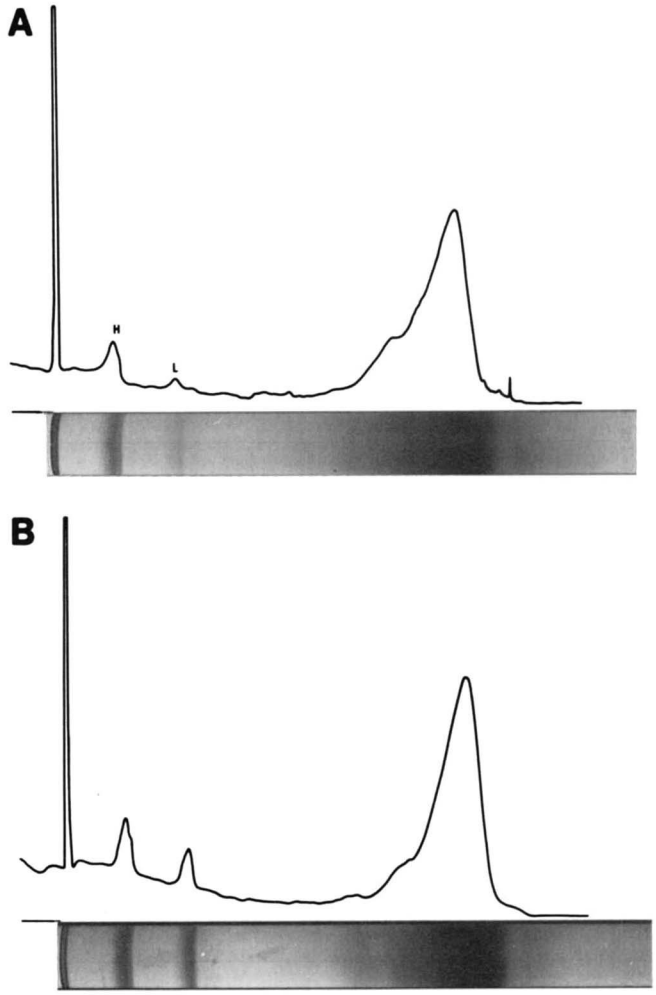

Fig. 6. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis utilizing gels of 3.5% acrylamide and densitometric scanning of the d < 1.210 g/ml fractions of adult (A) and old (B) male rats. Two major bands of apoB can be resolved, designated high molecular weight apoB (H) and low molecular weight apoB (L); 100 μg of apolipoprotein was applied to each gel.

[2] DeLamatre, J. G., and P. S. Roheim. Unpublished observations.

of a large amount of "free" apolipoproteins could be the result of an interference in this association as a result of *1*) reduced cholesterol efflux in old rats, due to a lower turnover rate, or *2*) an already "saturated" HDL in hyperlipidemic rats.

Synthesis of cholesterol by the liver appears to be decreased in the old rat (28–30). In addition, the turnover of plasma and tissue cholesterol appears to be reduced as well (4). Old rats excrete less cholesterol in the bile and feces (31), which may help to explain the diminished absorption of dietary cholesterol (28) due to an altered bile composition. The increased plasma concentrations of lipids (3–6, 8) and apolipoproteins would suggest that the uptake and utilization of lipoprotein-lipid by tissues is reduced to a greater extent than is synthesis. This may relate to the accumulation of apoA-IV in the "free" state, observed in old rats in our study.

We have shown that, in old rats, hyperlipidemia is associated with the following changes: *1*) a decreased VLDL concentration and increased IDL, LDL, and HDL concentrations; *2*) elevated plasma apolipoproteins involved with receptor-mediated uptake of lipoproteins, specifically apoB and apoE; and *3*) marked accumulation of "free" apolipoprotein A-IV. Future studies of how aging influences the mechanisms governing lipoprotein metabolism and to what extent endocrine functions are involved in these mechanisms would provide valuable information for understanding the control of lipid metabolism. Our observations may also serve as a useful basis for further studies toward understanding the mechanisms of hyperlipidemia associated with aging.

This research was supported by USPHS Grant 25596 and by USPHS Cardiovascular Training Grant HL07098B. The authors are grateful for the services of the Editorial Office of LSU Medical Center and for Yvonne Beck's help in the preparation of the manuscript.

Manuscript received 11 December 1981 and in revised form 10 May 1982.

REFERENCES

1. Adlersberg, D., L. E. Schaefer, A. G. Steinberg, and C. I. Wong. 1956. Age, sex, serum lipids, and coronary atherosclerosis. *J.A.M.A.* **162:** 619–622.
2. Bierman, E. L., and R. Ross. 1977. Aging and atherosclerosis. *In* Atherosclerosis Reviews. R. Paoletti and A. M. Gotto, Jr., editors. Raven, New York. 79–111.
3. Carlson, L. A., S. O. Froberg, and E. R. Nye. 1968. Effect of age on blood and tissue lipid levels in the male rat. *Gerontologia.* **14:** 65–79.
4. Hruza, Z., and M. Wachtlova. 1969. Decrease of cholesterol turnover in old rats. *Exp. Gerontol.* **4:** 245–250.
5. Dupont, J., A. A. Spindler, and M. M. Mathias. 1978. Dietary fat saturation and cholesterol and fatty acid synthesis in aging rats. *Age.* **1:** 93–99.
6. Reaven, G. M. 1978. Effect of age and sex on triglyceride metabolism in the rat. *J. Gerontol.* **33:** 368–371.
7. Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano, and N. Takeuchi. 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.* **19:** 544–552.
8. Malhotra, S., and D. Kritchevsky. 1978. The distribution and lipid composition of young and old rat plasma. *Mech. Ageing Dev.* **8:** 445–452.
9. Nilsson-Ehle, P., A. S. Garfinkel, and M. D. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49:** 667–693.
10. Brown, M. S., and J. L. Goldstein. 1976. Receptor-mediated control of cholesterol metabolism. *Science.* **191:** 150–154.
11. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
12. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.
13. Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57:** 714–721.
14. Roheim, P. S., and G. Vega. 1979. Electroimmunoassay of apolipoprotein A-I. *In* Report of the High Density Lipoprotein Methodology Workshop, San Francisco, CA. K. Lippel, editor. NIH Publication No. 79-1661. 241–251.
15. Shapiro, A. L., E. Vinuela, and J. V. Maizel, Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. *Biochem. Biophys. Res. Commun.* **28:** 815–820.
16. Bucolo, G., and H. David. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* **19:** 476–482.
17. Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. S. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Apoprotein content of plasma lipoproteins of the rat separated by gel chromatography or ultracentrifugation. *Biochem. Med.* **17:** 347–355.
20. Witztum, J. L., and G. Schonfeld. 1979. Lipoproteins in the plasma and hepatic perfusates of the Zucker fatty rat. *Diabetes.* **28:** 509–516.
21. Roth, G. S. 1979. Hormone action during aging: alterations and mechanisms. *Mech. Aging Dev.* **9:** 497–514.
22. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes. Metabolically distinct apoE and apoB, E receptors. *J. Biol. Chem.* **256:** 5646–5655.
23. Sparks, C. E., and J. B. Marsh. 1981. Metabolic heterogeneity of apolipoprotein B in the rat. *J. Lipid Res.* **22:** 519–527.
24. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma. Isolation and quantitation. *Eur. J. Biochem.* **99:** 333–343.

25. Green, P. H. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV. Intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
26. Roheim, P. S., L. Miller, and H. A. Eder. 1965. The formation of plasma lipoproteins from apoprotein in plasma. *J. Biol. Chem.* **240:** 2994–3001.
27. Fidge, N. H. 1980. The redistribution and metabolism of iodinated apolipoprotein A-IV in rats. *Biochim. Biophys. Acta.* **619:** 129–141.
28. Yamamoto, M., and Y. Yamamura. 1971. Changes in cholesterol metabolism in the aging rat. *Atherosclerosis.* **13:** 365–374.
29. Trout, E. C., K-Y. Taug Kao, C. A. Hizer, and T. H. McGavack. 1962. In vitro synthesis of free and ester cholesterol in various tissues of young and old rats. *J. Gerontol.* **17:** 363–368.
30. Rosenman, R. H., and E. Shibata. 1952. Effect of age upon hepatic synthesis of cholesterol in rat. *Proc. Soc. Exp. Biol. Med.* **81:** 296–298.
31. Hruza, S., and V. Zbuzkova. 1973. Decrease of excretion of cholesterol during aging. *Exp. Gerontol.* **89:** 29–37.