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Apolipoprotein B (B-48) of rat chylomicrons is not a precursor of the apolipoprotein of low density lipoproteins

(lipoprotein catabolism/lipoprotein remnant)

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ABSTRACT **We have found that, in chylomicrons from intestinal lymph fistulas in the rat, the sole molecular species of apolipoprotein B (apo B) is B-48. This protein is analogous to the B-48 apoprotein of human chylomicrons. In contrast, preparations of chylomicrons from blood serum are known to contain species of B apolipoproteins of higher molecular weight, presumably due to the presence of hepatogenous lipoproteins. We studied the removal of ^{125}I-labeled apo B-48 of intestinal lymph chylomicrons from blood plasma of rats. The removal of [^{14}C]cholesteryl esters of biologically labeled chylomicrons was unaffected by radioiodination. The labeled cholesteryl esters and apo B-48 disappeared rapidly from the density (ρ) <1.006 g/ml fraction of plasma. In contrast to the apo B of very low density lipoproteins, <1% of the ^{125}I-labeled B-48 was found in low density lipoproteins (1.019 < ρ < 1.063 g/ml) after 1 hr. No ^{125}I was found in the B-100 or B-95 apolipoproteins at any time. We conclude that, unlike the species of apo B found uniquely in hepatogenous very low density lipoproteins, the apo B-48 protein of chylomicrons is not a precursor of the B apoprotein of low density lipoproteins.**

In humans, most of the apolipoprotein B (apo B) of very low density lipoproteins (VLDL) is converted to low density lipoproteins (LDL) (1). By contrast, in the rat, 5% or less is so converted; the remainder is taken up by the liver as a VLDL remnant (2). Findings in a newly recognized human metabolic disorder, normotriglyceridemic abetalipoproteinemia (3) suggest that chylomicron apo B is not converted to typical LDL in humans. In this disorder, the apo B of normal VLDL and LDL, designated B-100, is lacking and no normal VLDL or LDL are present. However, chylomicrons are produced and metabolized normally. They contain an apo B of lower molecular weight, B-48 (3). B-48 is the major apo B of normal chylomicrons from human thoracic duct lymph (4).

In rats, the apo B of mesenteric lymph also has a lower molecular weight than that of LDL (5). In addition to this protein, preparations of chylomicrons separated from rat serum contain two apo B species of higher molecular weight, presumably due to contamination with lipoproteins of hepatic origin (6). All three of these elements are present in rat LDL (6).

We have found that apo B-48 is the sole B protein species of mesenteric lymph chylomicrons in the rat. To determine whether the protein moiety of LDL can arise from chylomicron precursors as it does from VLDL, we studied the fate, in intact rats, of the apo B-48 of ^{125}I-labeled rat mesenteric lymph chylomicrons. We report here that the apo B-48 of LDL is not derived from the protein moiety of chylomicrons, nor does the chylomicron protein contribute to the formation of the higher molecular weight species of apo B in LDL. We have found B-48, as well as the higher molecular weight species of apo B, in VLDL isolated from perfusates of rat livers. The B-48 of rat LDL may, therefore, be largely of hepatic origin.

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METHODS

Preparation and Labeling of Chylomicrons. Small chylomicrons were isolated from rat mesenteric lymph during intraduodenal infusion of 10% glucose in saline (7) containing [^{3}H]cholesterol (250 μCi; 1 Ci = 3.7×10^{10} becquerels) (8). The small chylomicrons were labeled with ^{125}I by the iodine monochloride method (9). Typically, 4 ml of chylomicrons, containing 4 mg of protein, were mixed with 800 μl of 1 M glycine buffer (pH 10.0), 2 mCi of ^{125}I, and 200 μl ICl (0.4 mM in 2 M NaCl) for 5 min. Unreacted ^{125}I was removed on Sephadex G-50 (Pharmacia, AB); chylomicrons were eluted with Krebs–Henseleit buffer. Efficiency of labeling was ≈35%. Less than 1% of the ^{125}I was soluble in trichloracetic acid and 47–56% was soluble in ethanol/ether, 3:1 (vol/vol). The labeled chylomicrons were incubated with VLDL-free serum (0.67 mg of triglycerides per ml of serum) for 1 hr at 23°C to remove labeled non-B apoproteins by exchange with high density lipoproteins (HDL) (7). They were then isolated by centrifugation at 4°C for 2.6×10^{7} g_{avg} min. These chylomicrons were dialyzed for 2 hr at 6°C against Krebs–Henseleit buffer.

Reinjection of Labeled Chylomicrons. Labeled chylomicrons were injected during brief ether anesthesia into femoral veins of rats fasted for 24 hr. In some experiments, 0.2-ml blood samples were obtained at intervals from a tail vein. All rats were anesthetized at specified times and exsanguinated from the abdominal aorta. The blood was placed on ice and the serum was separated at 4°C. The livers were weighed and frozen.

Isolation of Lipoproteins and Measurement of ^{125}I. Gentamycin sulfate (0.1 mg/ml) and EDTA (1 mM) were added to serum and all solutions. Lipoproteins were separated sequentially in a 40.3 rotor of a Beckman ultracentrifuge at density (ρ) = 1.006, 1.019, 1.040, and 1.063 g/ml for $10^{8}g_{avg}$ min. Densities were adjusted with $^{2}H_{2}0$ in 0.15 M NaCl. Lipoproteins were delipidated with ethanol/diethyl ether (10). One portion of the solubilized protein was assayed directly for ^{125}I and another, containing no more than 20 μg of protein, was subjected

Abbreviations: VLDL, LDL, and HDL, very low density, low density, and high density lipoproteins of serum, respectively; apo B, apolipoprotein B; ρ, density.

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to electrophoresis (10). After the tracking dye had traveled 6 cm, the gels were fixed in methanol/acetic acid/water (9:2:9) for 30 min and then soaked in 10% acetic acid/water to clarify the background. Bands of fixed protein were defined by light scattering from a Tyndall beam to permit sectioning of the gels for measurement of radioactivity. Electrophoretic mobilities of the B apolipoproteins were determined on separate samples as described (4). Mesenteric lymph chylomicrons contained only one species of apo B. Its mobility on electrophoresis in $NaDodSO_4$ corresponded exactly with the B-48 protein of human chylomicrons. Two species of higher molecular weight apo B were observed, corresponding to B-100 and B-95 in the centile system used to characterize human apolipoprotein B (4). Of these, B-100 appeared to be the dominant species. The gels were cut into five sections for assay of ^{125}I: (*i*) origin, (*ii*) apo B-100/B-95, (*iii*) the area between apo B-100/B-95 and B-48, (*iv*) apo B-48, and (*v*) proteins migrating ahead of apo B-48 (E and C apoproteins). All solvents used to extract lipids and portions of liver were also assayed for ^{125}I. Recovery of ^{125}I added to the gels was 85.7 ± 1.6%. Protein was determined by a modification of the method of Lowry, with bovine serum albumin as standard (11). Triglycerides were estimated by an automated procedure (12). [^{3}H]Cholesteryl esters were separated in lipid extracts by thin-layer chromatography and estimated as described (13).

RESULTS

All lipoprotein fractions from blood serum contained components having the mobilities of B-48 and B-100/B-95 (Fig. 1). The proportion of B-100/B-95 relative to that of B-48 increased with increasing density. B-48 was present in chylomicrons of intestinal lymph, but no B-100 or B-95 could be detected. B-48 was also found, together with B-100, in VLDL isolated from perfusates of rat livers.

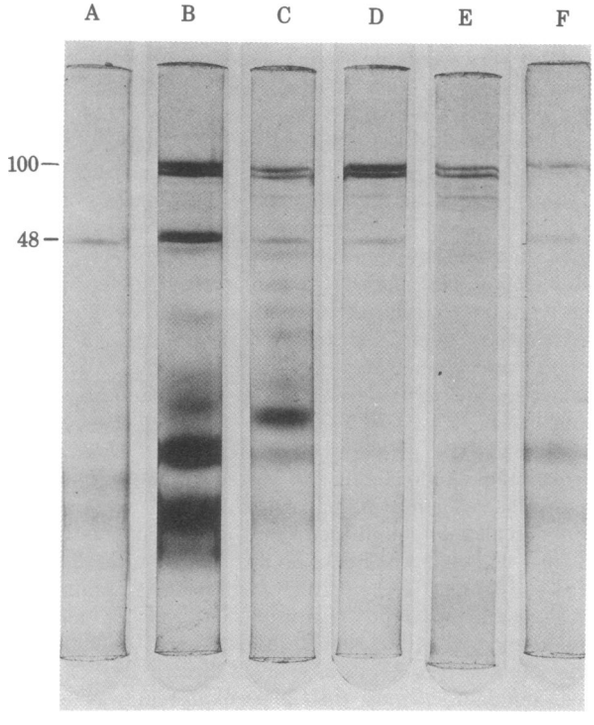

FIG. 1. Electrophoretograms of apolipoproteins in 3% polyacrylamide/$NaDodSO_4$ (Coomassie blue staining) (4). Lanes: A, intestinal lymph chylomicrons; B–E, serum of fasted rats—B, $\rho < 1.006$ g/ml; C, $1.006 < \rho < 1.019$; D, $1.019 < \rho < 1.040$; E, $1.040 < \rho < 1.063$; F, $\rho < 1.006$ portion of liver perfusate (15) obtained after preperfusion for 1 hr. 100 and 48, Positions of B-100 and B-48, respectively.

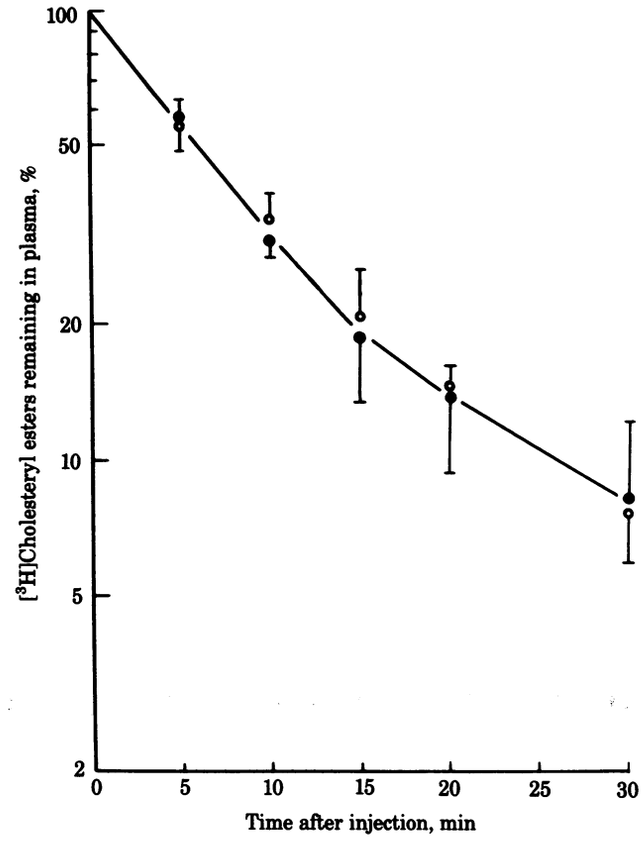

FIG. 2. Removal from blood plasma of [^{3}H]cholesteryl esters of chlomicrons labeled biologically with [^{3}H]cholesterol (○) or labeled biologically with [^{3}H]cholesterol and subsequently with ^{125}I by the iodine monochloride method (●). Results (mean ± SD for triplicate experiments) are calculated as percent of that present on initial mixing of chylomicrons in blood plasma, taking plasma volume as 4.5% of body weight.

To evaluate the effect of radioiodination on the metabolism of chylomicrons, a portion of [^{3}H]cholesterol-labeled chylomicrons was iodinated. When the singly labeled and doubly labeled chylomicrons were injected into rats, the [^{3}H]cholesteryl esters were removed from the blood plasma at virtually identical rates (Fig. 2). Fifteen minutes after injection, 54.3 ± 9.7% and 57.8 ± 8.3% of the [^{3}H]cholesteryl esters were recovered in the livers of animals injected with singly and doubly labeled chylomicrons, respectively. ^{125}I-labeled B-48 of chylomicrons was removed somewhat more slowly from $\rho < 1.006$ g/ml lipoproteins than were [^{3}H]cholesteryl esters (Fig. 3).

When ^{125}I-labeled chylomicrons were added to serum in a concentration equivalent to that occuring immediately after injection of chylomicrons into rats, only a small fraction of B-48 was recovered in lipoproteins of higher density obtained by sequential ultracentrifugation (Table 1, 0 time sample). After injection of chylomicrons into rats, small, but proportionately larger, amounts of B-48 appeared in the other lipoprotein fractions (Table 1). The values were maximal 15 min after injection but, by 60 min, negligible amounts of ^{125}I (<1%) remained in B-48 in any lipoprotein fraction. The mean value for LDL_2 after 60 min (0.045%) was significantly less than the 15-min value (0.43%) ($P < 0.01$). No detectable amounts of ^{125}I were found in apo B-100 in any fraction at any time.

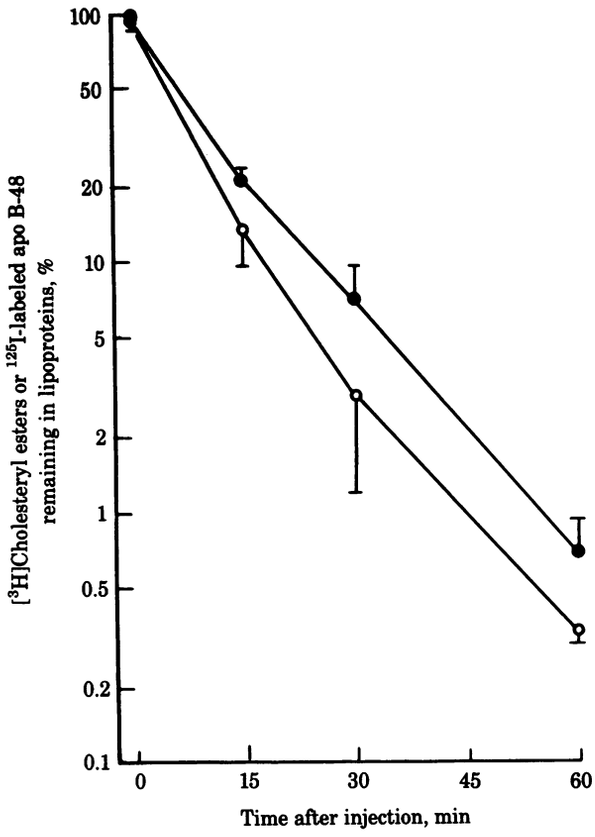

FIG. 3. Removal of [^{3}H]cholesteryl esters (○) and ^{125}I-labeled B-48 (●) from $\rho < 1.006$ g/ml lipoproteins after intravenous injection of chylomicrons. Results are calculated as in Fig. 2 and represent mean ± SD for triplicate experiments with [^{3}H]cholesteryl ester-labeled chylomicrons and triplicate experiments with ^{125}I-labeled chylomicrons.

Only small amounts of [^{3}H]cholesteryl esters appeared in $\rho > 1.006$ g/ml lipoproteins. Maximal values were 1.7% in intermediate density lipoproteins and 0.6% in LDL_1 15 min after injection, 0.4% in LDL_2 30 min after injection, and 2.2% in HDL ($\rho > 1.063$ g/ml) 60 min after injection. Mean recovery of cholesteryl esters in these fractions was 96.7 ± 2.0%.

DISCUSSION

We found the mobility of the lower molecular weight species of rat apo B in $NaDodSO_4$ to be identical with that of B-48 of human chylomicrons (4). Thus, it seems probable that these proteins are homologous. Likewise, we found the largest apo B species of VLDL and LDL in blood plasma to have an apparent molecular weight identical to that of the apo B of human plasma VLDL and LDL on electrophoresis in $NaDodSO_4$ (4).

Much less of the B protein of plasma VLDL appears to give rise to apo B of LDL in the rat than in humans (2, 6). Elovson *et al.* (6) have shown appreciable labeling in LDL of both the B-48 species and the elements of larger apparent molecular weight after the administration of labeled plasma VLDL, consistent with the labeling of apo B of LDL reported by us earlier; that is, the content of both radiolabeled B-48 and B-100 was found to increase progressively over a 60-min period after the injection of labeled VLDL (2). In contrast, our data indicate that only minimal amounts of labeled B-48 protein from chylomicrons appear in $\rho > 1.006$ g/ml lipoproteins. Furthermore, B-48 of chylomicron origin disappears much more rapidly from these lipoproteins than the label derived from VLDL B proteins (Table 1). These results suggest that the small amount of labeled B-48 in these fractions represents protein in chylomicron remnants, which are known to turn over rapidly, rather than in LDL, which have a much lower turnover rate. Elovson *et al.* (6) reported that the smaller molecular weight species of LDL apo B derived from VLDL turned over somewhat more rapidly than the species of high molecular weight and concluded that these species exist on different LDL particles. However, plasma VLDL contain particles originating in intestine as well as liver, and B-48 evidently is secreted in both hepatogenous VLDL and intestinal chylomicrons. The kinetic behavior of B-48 derived from plasma VLDL in that study may therefore have reflected the composite behavior of two distinct populations of lipoproteins. In experiments in which cholesterylester-labeled plasma VLDL were injected into functionally hepatectomized rats, an appreciable amount of labeled cholesteryl ester(s) appeared in LDL (14), consistent with the presence of small amounts of remnant lipoproteins in the LDL density interval. As in those experiments, some of the labeled cholesteryl esters in lipoproteins of higher density may have been derived from the action of lecithin–cholesterol acyltransferase on labeled free cholesterol in HDL (15).

Our results are therefore consistent with the conclusion that the apo B of chylomicrons is not a precursor of apo B of typical LDL. Schaefer *et al.* (16) have reported that ≈18% of apo B is in LDL after intravenous injection of a sample of radioiodinated chylomicrons obtained from human chylous pleural fluid. As chylomicrons in human thoracic duct lymph contain B-100 as well as B-48 (4), it is likely that their apo B included hepatogenous apo B (B-100), which would be expected to be converted to B-100 of LDL.

We conclude that, in the rat, probably unlike the human, B-48 is synthesized in both liver and intestine, whereas B-100 is synthesized only in the liver. Unlike the hepatogenous lipoproteins that give rise to LDL, the products of catabolism of chylomicrons are removed rapidly from plasma without generating persistent apo B-containing lipoproteins.

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Table 1. Distribution of B-48 in serum lipoprotein fractions after intravenous injection of chylomicrons

Fraction	B-48, % of injected			
	0 min	15 min	30 min	60 min
VLDL ($\rho < 1.006$)	92 ± 4.8	21 ± 5.6	7.2 ± 4.5	0.70 ± 0.42
IDL ($1.006 < \rho < 1.019$)	3.1 ± 2.7	6.0 ± 2.0	4.2 ± 3.4	0.73 ± 0.17
LDL_1 ($1.019 < \rho < 1.040$)	1.2 ± 1.0	1.7 ± 2.1	1.6 ± 2.2	0.52 ± 0.83
LDL_2 ($1.040 < \rho < 1.063$)	0.29 ± 0.46	0.43 ± 0.53	0.25 ± 0.27	0.045 ± 0.06*

Zero time results were obtained after addition of chylomicrons to serum *in vitro*. Other results are based on initial mixing of chylomicrons in blood plasma, taken as 4.5% of body weight. Results are mean ± SD of four experiments. IDL, intermediate density lipoproteins.

* Difference between mean levels at 15 min and 60 min is significant ($P < 0.01$, two-tailed t test) after logarithmic transformation to normalize the distribution of values.

1. Sigurdsson, G., Nicoll, A. & Lewis, B. (1975) *J. Clin. Invest.* **56,** 1481–1490.
2. Faergeman, O., Sata, T., Kane, J. P. & Havel, R. J. (1975) *J. Clin. Invest.* **56,** 1396–1403.
3. Malloy, M. J., Kane, J. P., Hardman, D., Hamilton, R. L. & Dalal, K. B. (1981) *J. Clin. Invest.* **67,** 1441–1450.
4. Kane, J. P., Hardman, D. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2465–2469.
5. Krishnaiah, K. V., Walker, L. F., Borensztajn, J., Schonfeld, G. & Getz, G. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3806–3810.
6. Elovson, J., Huang, Y. O., Baker, N. & Kannan, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 157–161.
7. Imaizumi, K., Fainaru, M. & Havel, R. J. (1980) *J. Lipid Res.* **19,** 712–722.
8. Windler, E., Chao, Y. S. & Havel, R. J. (1980) *J. Biol. Chem.* **255,** 5475–5480.
9. McFarlane, A. S. (1958) *Nature (London)* **182,** 53.
10. Hardman, D. & Kane, J. P. (1980) *Anal. Biochem.* **105,** 174–180.
11. Kane, J. P., Sata, T., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56,** 1622–1634.
12. Rush, R., Leon, L. & Turrell, J. (1970) *Advances in Automated Analysis-Technicon International Congress*, ed. Barton, E. C. (Media, Inc., Tarrytown, New York), Vol. 1, pp 503–507.
13. Fielding, C. J. (1979) *Biochim. Biophys. Acta* **573,** 255–265.
14. Mjøs, O. D., Faergeman, O., Hamilton, R. L. & Havel, R. J. (1975) *J. Clin. Invest.* **56,** 603–615.
15. Hamilton, R. L., Williams, M. C., Fielding, C. J. & Havel, R. J. (1976) *J. Clin. Invest.* **58,** 667–680.
16. Schaefer, E. J., Jenkins, L. L. & Brewer, H. B., Jr. (1978) *Biochem. Biophys. Res. Commun.* **80,** 405.