Metabolic heterogeneity of apolipoprotein B in the rat

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Abstract Triglyceride-rich lipoprotein apoprotein catabolism was studied in rats from 5 to 60 min after intravenous injection of ^{125}I-labeled lipoproteins. The plasma and liver labeled apoprotein content was analyzed by gel filtration column chromatography using an elution buffer containing 1% sodium dodecyl sulfate. The method resolved two B apoproteins of lower (apo B_l) and higher (apo B_h) molecular weight. Total apoprotein B disappeared from plasma faster than either apo E or apo C and the smaller sized apo B_l had the most rapid disappearance, with 90% being lost after 60 min. The larger sized apo B_h disappeared rapidly from the plasma in the first 15 min but between 15 and 60 min 40% of the apo B_h remained in the plasma, associated with low density lipoprotein. Apoprotein analysis of liver homogenates was consistent with the plasma results. There was 28% of apo B_l compared to 16% of apo B_h present in the liver 5 min after injection, expressed as percent of initial injected radioactivity in each fraction. Apo B_l and apo B_h were the predominant liver apoproteins up to 30 min but by 60 min there was little of either apo B in the liver. In contrast to apo B, there was a relatively constant amount of apo E and apo C, about 10%, associated with the liver over 60 min. Plasma apo E declined progressively to 68% and apo C to 86% of initial concentration by 60 min. These findings suggest that there is differential hepatic catabolism of a subpopulation of triglyceride-rich lipoproteins containing apo B_l. A population of triglyceride-rich lipoproteins containing apo B_h preferentially enters the low density lipoprotein pool with a slower catabolism. The results are consistent with an hypothesis that apo B_l mediates binding and rapid hepatic catabolism of its associated lipoproteins. Metabolic heterogeneity of the triglyceride-rich lipoproteins may be explained by the molecular heterogeneity of apoprotein B.—**Sparks, C. E., and J. B. Marsh.** Metabolic heterogeneity of apolipoprotein B in the rat. *J. Lipid Res.* 1981. **22:** 519–527.

Supplementary key words apo B metabolism · triglyceride-rich lipoprotein · chylomicrons · lipoprotein metabolism · hepatic lipoprotein metabolism

Triglyceride-rich lipoprotein (TRL) is synthesized by the liver and intestine (1–5) and contains both very low density lipoprotein (VLDL) and chylomicrons (6). Nascent chylomicrons from the intestine contain apo A-I (7) but circulating chylomicrons are similar to VLDL in that their surface is enriched in apo C and phospholipid (7–9). Apparently, this surface apo C inhibits hepatic recognition of lipoproteins for uptake and degradation (10–12). The TRL serves as substrate for extrahepatic lipoprotein lipase which converts it to intermediate or remnant particles that are depleted in surface components (6, 13–16). The TRL remnant particles then become recognizable for hepatic uptake and catabolism (17–21). Recent evidence suggests that apo E participates in the hepatic recognition process for lipoproteins (10–12, 22, 23). A role for apo B in hepatic lipoprotein recognition has not been established.

In man, a large portion of TRL enters the low density lipoprotein (LDL) pool and LDL formation from VLDL follows a precursor–product relationship (24, 25). The formation of LDL follows the action of triglyceride-lipase enzymes on TRL but the relative role of extrahepatic lipoprotein lipase and hepatic-triglyceride lipase in LDL formation has not been elucidated (26). In rats, the formation of LDL is less clearly understood. Most of TRL is cleared by the liver and only a small portion enters the pathway to LDL formation (21). Kinetic studies using injection of labeled VLDL indicate that in the rat there may be direct entry of synthesized lipoprotein into the LDL density class (27) and direct synthesis of LDL has been demonstrated in liver perfusion (5). Factors influencing entry of lipoproteins into LDL are not known but in man and rat two distinct apo B proteins have been identified (28, 29). Each apo B could specifically direct its associated particle to either the hepatic clearance or LDL pathways. The current study demonstrates that rat apo B is not only heterogeneous in molecular size but also in its metabolism. The results indicate that the smaller apo B_l directs TRL-derived

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Abbreviations: TRL, triglyceride-rich lipoprotein (d < 1.006 g/ml containing chylomicrons); VLDL, very low density lipoprotein (d < 1.006 g/ml); LDL, low density lipoprotein (1.006 < d < 1.063 g/ml); HDL, high density lipoprotein (1.063 < d < 1.225 g/ml); SDS, sodium dodecyl sulfate: apo B_h, apolipoprotein B of higher molecular weight; apo B_l, apolipoprotein B of lower molecular weight; PAGE, polyacrylamide gel electrophoresis.

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particles to the liver for catabolism and the larger apo B_h favors entry of its associated particle into the LDL pool.

MATERIALS AND METHODS

Rats were anesthetized by intraperitoneal injection of 30 mg/kg of nembutal and were injected intravenously with ^{125}I-labeled TRL via an exposed saphenous vein. An average of 0.28 ml of TRL in 0.15 M NaCl containing 2 mM EDTA at pH 7.4 was injected containing an average of 525 μg of apoprotein and 4.5×10^6 dpm of radioactivity. At the designated time after injection, 5 ml of blood was obtained by aortic puncture and collected in a heparinized syringe containing 200 μl of heparin solution (Armour Pharmaceutical Company, Phoenix, AZ). Triplicate 0.2-ml plasma samples were assayed for radioactivity and total plasma radioactivity was determined by multiplying the average dpm/ml times the plasma volume assumed equal to 4.2% of the body weight (30). All experiments were performed between 9 and 11 AM on animals fed laboratory chow ad libitum.

Preparation of triglyceride-rich lipoproteins

Lipoproteins were isolated from the sera of male rats of the Fischer 344 strain weighing 200–300 g. The rats were fed for 5–10 days on a diet containing 68% (w/w) sucrose, 10% (w/w) vegetable oil, and 18% (w/w) casein (ICN, Nutritional Biochemicals, Cleveland, OH). The rats were lightly anesthetized by intraperitoneal injection of 30 mg/kg nembutal and blood was obtained by aortic puncture at 9–11 AM from animals fed ad lib. Lipoprotein fractions were prepared by ultracentrifugation of pooled sera as previously described (5, 31). The TRL fraction (d $<$ 1.006 g/ml) contained both chylomicrons and VLDL. At a concentration of 2–4 mg/ml protein, ^{125}I-labeled TRL was prepared using the McFarlane-ICL method as described (32) using the modification of Bilheimer, Eisenberg, and Levy (33). The ^{125}I-labeled TRL has less than one iodine atom per molecule of apoprotein assuming an apoprotein molecular weight of 2×10^5 and specific activities ranged from 1–10 dpm/ng protein. An average of 18% (range, 6–35%) of the radioactivity was associated with lipid. In some experiments, total lipoproteins were isolated at d $<$ 1.21 g/ml and lipoproteins were separated by gel filtration chromatography on 1.5 × 90 cm columns of Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ) by using our modification (5) of the method of Rudel et al. (34).

Preparation of samples for column analyses

Delipidated plasma, whole plasma, and isolated lipoprotein fractions were prepared for column analysis as described elsewhere (35). Plasma containing ^{125}I-labeled lipoproteins was delipidated using the method of Lux, John, and Brewer (36) as described previously (5, 31). To delipidated plasma precipitates, up to 0.2 ml plasma, 0.5 ml of 10% SDS buffer consisting of 0.1 M Tris (hydroxy methyl) aminomethane, pH 7.4, containing 10% (w/v) SDS, 10% (v/v) glycerol, and 10% (v/v) 2-mercaptoethanol was added. The tubes were tightly sealed and incubated at room temperature for 18 hr. The samples were then heated at 100°C for 10 min with vigorous mixing. Whole plasma, containing the ^{125}I-labeled lipoproteins, was prepared for column analysis by dilution with an equal volume of 10% SDS buffer, heating for 3–5 min at 100°C, and cooling to room temperature. The lipid radioactivity was determined by measuring radioactivity in extracted lipid or by the difference between delipidated plasma and whole plasma radioactivity. Labeled, ultracentrifugally-prepared or column-isolated lipoprotein fractions were prepared for SDS-column analysis by dilution with an equal volume of 10% SDS buffer, heating for 3–5 min at 100°C, and cooling to room temperature.

At the conclusion of the experimental period, livers were perfused via the portal vein for 3 min with 0.15 M NaCl at room temperature, a procedure found to remove more than 98% of contaminating plasma radioactivity. The livers were weighed and immediately frozen using dry ice. The frozen liver was added to 3 ml of 0.15 M NaCl per g wet weight containing dextran T-500 (Pharmacia Fine Chemicals, Piscataway, NJ), 0.5 g per 10 g liver. The mixture was quickly homogenized with a Polytron (Brinkmann Instruments, Westbury, NY). One ml of homogenate was delipidated similarly to plasma and treated in the same way as delipidated plasma, except that the precipitate was dissolved in 3 ml of 10% SDS buffer and, just prior to application to the column, high molecular weight nucleic acids were removed by ultracentrifugation for 30 min at 15°C at 30,000 rpm in the Beckman L3-50 ultracentrifuge using the 40.3 rotor.

Apoprotein separation and analysis

Labeled apoproteins were separated on 165 × 1.5 cm glass columns (Bio-Rad Laboratories, Richmond, CA) containing Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated in a column buffer containing 0.1 M sodium phosphate, pH 7.4, and 1% (w/v) SDS as described previously (35). Up to

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2.0 ml of sample was applied to the column and apoproteins were eluted at an average flow rate of 7–8 ml/hr. Fractions were collected at 30-min intervals and assayed for radioactivity. Column recovery was uniformly greater than 95% of the applied radioactivity.

Radioactivity measurements

Radioassays of ^{125}I-labeled lipoproteins and column fractions of ^{125}I-labeled apoproteins were made in a Searle 1144 gamma scintillation spectrometer with a counting efficiency of 82%. Radioassays of ^{3}H-labeled lipoproteins were made in a Packard Tri-Carb liquid scintillation spectrometer. Counting errors in all cases were less than 5%. All results are expressed as mean ± S.E. The Student *t* was used to calculate significance of differences between results.

RESULTS

Characterization of ^{125}I-labeled TRL

TRL was isolated and labeled with ^{125}I as described. The labeled TRL was added to plasma and delipidated with chloroform–methanol–ether. Whole and delipidated plasma ^{125}I-labeled apoprotein preparations were analyzed by SDS-gel filtration column chromatography and radioactive peaks were separated and identified as a high molecular weight apo B_h, a low molecular weight apo B_l, apo E, apo C, ^{125}iodide, and ^{125}I-labeled peptides as described previously (35) and seen in **Fig. 1A.** Identification of the apo B peaks was based on insolubility in 50% isopropanol, analysis by SDS-PAGE, and amino acid analysis, as reported elsewhere (35). The radioactivity distributions of six preparations of ^{125}I-labeled TRL is summarized in **Table 1** (zero time).

Change in plasma apoprotein distribution after ^{125}I-labeled TRL injection

Freshly labeled ^{125}I-labeled TRL was injected intravenously into rats and blood was obtained either 5 or 60 min after injection by aortic puncture. Plasma was prepared and the delipidated plasma radioactivity was analyzed by SDS-column chromatography. The results of a representative experiment are shown in Fig. 1. As seen in the figure, virtually none of the apo B_l present in the injected ^{125}I-labeled TRL was detectable in the 60-min plasma samples. The average percent distribution of plasma radioactivity 5 and 60 min after injection of ^{125}I-labeled TRL found in several experiments is summarized in Table 1. The major difference between the initial TRL and the 5-min plasma distribution was the reduction of the percent of plasma radioactivity in the apo B_l. The decline in the apo B_l resulted in halving of the apo B_l/apo B_h ratio. At 60 min, the percentage of apo B_h had declined, along with apo B_l.

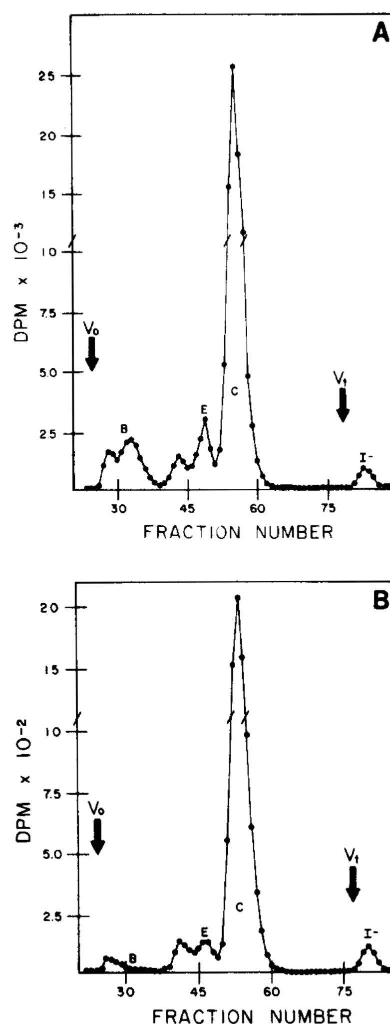

Fig. 1. SDS-gel filtration column chromatography of solubilized ^{125}I-labeled apoproteins of plasma. The results in Fig. 1A represent SDS-column analysis of ^{125}I-labeled TRL added to rat plasma and in Fig. 1B represent SDS-column analysis of plasma 60 min after ^{125}I-labeled TRL injection into rats. TRL was obtained from sucrose-fed rats and radioiodinated as described in Methods. The void volume (V_0) and total volume (V_t), iodide exclusion volume (I^-) and the exclusion volumes of apo B (B), apo E (E), and apo C (C), are indicated above the appropriate peaks. The radioactivity in dpm is plotted against fraction number with a flow of 3.87 ml/fraction and 4.02 ml/fraction in the columns of 1A and 1B, respectively, and the fractions were collected at 30-min intervals. Note that there are two apo B peaks. The peak collected in fraction 25–30 represents apo B_h and the peak in fractions 30–37 represents apo B_l.

Hepatic apoprotein distribution after ^{125}I-labeled TRL injection

The ^{125}I-labeled apoprotein distribution in the livers at 5 and 60 min after ^{125}I-labeled TRL injection was

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TABLE 1. Plasma and liver distribution of ^{125}I-labeled apoproteins at 5 and 60 minutes after injection of ^{125}I-labeled TRL[a]

	Time	B_h	B_l	B_{total}	$\frac{B_l}{B_h}$	E	C	I-Peptide
^{125}I-TRL, n = 6	0	3.98 ± 0.24	8.01 ± 0.40	11.99 ± 0.40	2.09 ± 0.20	7.19 ± 0.81	69.69 ± 1.49	5.24 ± 1.34
Plasma, n = 9	5 min	3.96 ± 0.30	3.90 ± 0.34[b]	7.86 ± 0.54[b]	1.01 ± 0.08[b]	8.14 ± 0.56	74.66 ± 1.00	3.41 ± 0.58
Plasma, n = 8	60 min	1.86 ± 0.22[b]	1.48 ± 0.29[b]	3.33 ± 0.35[b]	0.89 ± 0.24[b]	6.80 ± 0.77	78.04 ± 1.54	4.81 ± 0.77
Liver, n = 8	5 min	5.23 ± 0.39	18.49 ± 0.97[b]	23.71 ± 1.22[b]	3.62 ± 0.24[b]	8.31 ± 0.75	57.13 ± 1.72	3.40 ± 0.35
Liver, n = 5	60 min	1.87 ± 0.26[b,c]	2.15 ± 0.29[b,c]	4.02 ± 0.50[b,c]	1.20 ± 0.14[b,c]	5.05 ± 0.26	75.51 ± 1.83	10.46 ± 1.14[b,c]

[a] The distribution of ^{125}I-radioactivity was determined by SDS-gel filtration column chromatography of plasma and liver homogenates following injection as described in Methods. The results are expressed at each time point as the average percent of total label applied to the column present in each fraction. At 5 min, liver contained 24% apo B in contrast to 60 min where liver contained only 4% apo B. The recovery of label into ultracentrifugal supernatants was 86.9 ± 4% and 88.1 ± 3.0%, respectively, at these times with the remainder of the label contained in the pellet containing nucleic acids. The differences in apo B content did not affect solubility in SDS-buffer prior to column analysis.

[b] Significantly different from the corresponding values for the injected ^{125}I-labeled TRL ($P < 0.05$).

[c] Significantly different from the corresponding value at 5 min ($P < 0.01$).

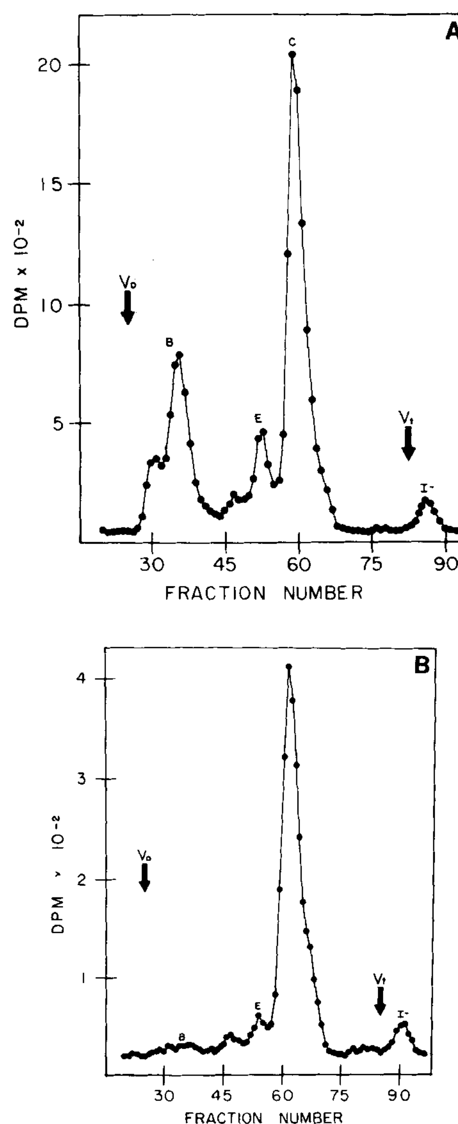

Fig. 2. SDS-gel filtration column chromatography of solubilized ^{125}I-labeled apoproteins of liver homogenates 5 min (A) and 60 min (B) after injection of ^{125}I-labeled TRL into rats. The peaks are identified as in Fig. 1 and the columns were run identically except that the flow was 3.74 ml/fraction in 2A and 3.49 ml/fraction in 2B. In Fig. 2A, apo B_h appeared in fractions 28–33 and apo B_l in fractions 34–41. In Fig. 2B, there was little radioactivity in the apo B region.

also analyzed by SDS-column chromatography. As seen in **Fig. 2A,** after 5 min a large portion of hepatic apo B was composed of apo B_l. After 60 min (Fig. 2B), there was very little apo B of either type present and apo C constituted most of the hepatic radioactivity. The average percent distributions of hepatic radioactivity 5 and 60 min after injection of ^{125}I-labeled TRL in five to eight experiments are summarized in Table 1. As seen, the 5-min hepatic radioactivity distribution differs markedly in apo B composition from

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either the initial ^{125}I-labeled TRL or the 5-min plasma. After 5 min, approximately one-fourth of hepatic radioactivity was labeled apo B, 75% of which was apo B_l. The preponderance of apo B_l in the liver was reflected in a ratio of apo B_l/apo B_h of 3.62 compared to 1.01 in the plasma at the same time. After 60 min, only 4% of hepatic radioactivity was in the apo B region of the chromatogram while the predominant contributors to hepatic radioactivity were apo C, labeled peptides, and ^{125}iodide.

Time course of ^{125}I-labeled apoprotein disappearance from plasma and appearance in liver

In one set of experiments, after injection of ^{125}I-labeled TRL into eight nonfasting recipient rats, plasma and hepatic ^{125}I-labeled apoprotein radioactivity was analyzed 5, 15, 30, and 60 min after injection (two rats at each time point). The plasma apoprotein radioactivity remaining expressed as a percent of the initial dose of each plasma ^{125}I-labeled apoprotein was plotted against time (**Fig. 3**). After 5 min, roughly 5–10% of labeled apo C, apo E, and apo B_h had been removed from the plasma compartment. In contrast, over 50% of the apo B_l had been removed by this time. The sizeable loss of apo B_l accounted for the loss of total apo B radioactivity. During the next 10 min there was rapid loss of radioactivity in both fractions of apo B. Unlike apo B, there was a gradual decline in plasma apo C and apo E radioactivity throughout the 60-min time period. Labeled plasma total apo B and apo B_l declined slowly over the next 45 min, whereas the apo B_h remained at a relatively constant concentration amounting to about 50% of the initial apo B_l radioactivity over this time period. These results suggest that during early TRL metabolism there is a change in the composition of plasma apo B subfractions due to the rapid and preferential removal of the smaller apo B. As a result there is loss of 80% of the smaller apo B_l and 50% of the large apo B_h after 15 min of circulation. The rapid phase is followed by a much slower phase of apo B metabolism where the larger apo B_h predominates in plasma.

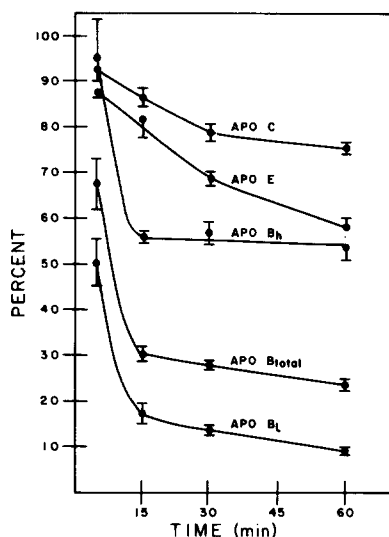

Fig. 3. Time course of the disappearance of individual labeled apoproteins of injected ^{125}I-labeled TRL from rat plasma. Values are expressed as the percent of initial plasma ^{125}I-labeled apoprotein present at the given time point. Each point represents averages of SDS-column analyses of delipidated and non-delipidated plasma of two individual rats amounting to four column analyses per time point. The SE of the four column analyses is indicated by the brackets. Each apoprotein decay curve is designated above the curve as apo C, apo E, apo B_h apo B_{total}, and apo B_l to identify individual apoproteins disappearance. The values were calculated as described in Table 2.

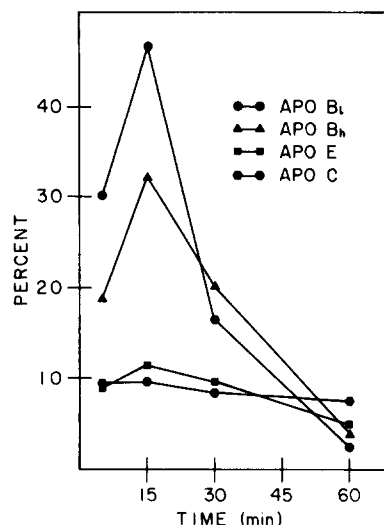

Fig. 4. Time course of the appearance of individual labeled apoproteins of injected ^{125}I-labeled TRL in the liver. Values are calculated as the percent of initial injected plasma ^{125}I-labeled apoproteins present in the liver at the given time point as described in Table 2. Each point represents the average value of two individual rat liver analyses. Individual apoprotein hepatic appearance curves are indicated as apo B_l (● —— ●), apo B_h (▲ —— ▲), apo E (■ —— ■), and apo C (● —— ●).

In corresponding livers, the hepatic apoprotein radioactivity as a percent of the initial plasma ^{125}I-labeled apoprotein (0 min) radioactivity was plotted against time (**Fig. 4**). After 5 min, roughly 10% of labeled plasma apo C and apo E were found in the liver and their levels tended to remain relatively constant over the 60-min time period. Approximately 30% and 19% of plasma apo B_l and apo B_h, respectively, were found in the liver 5 min after injection. Hepatic apo B_l and apo B_h continued to rise during the next 10 min and after 15 min represented 47% and 32% of their initial plasma radioactivities, respectively. Subsequently, hepatic content of both B apo-

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TABLE 2. Percent of injected apoprotein radioactivity present in plasma and liver 5 and 60 min after injection of ^{125}I-labeled TRL[a]

	Time	B_h	B_l	B_{total}	$\frac{B_l}{B_h}$	E	C
				Percent			
Plasma, n = 8	5 min	91.75 ± 7.24	52.85 ± 3.52	66.87 ± 4.65	0.585 ± 0.024	93.34 ± 3.81	95.66 ± 2.68
Plasma, n = 8	60 min	38.73 ± 5.27	11.48 ± 1.74	20.01 ± 2.43	0.312 ± 0.036	68.36 ± 3.76	85.85 ± 3.69
Liver, n = 8	5 min	15.75 ± 1.32	28.11 ± 1.86	23.95 ± 1.62	1.833 ± 0.134	12.41 ± 1.35	9.47 ± 0.62
Liver, n = 5	60 min	3.66 ± 0.62	2.16 ± 0.22	2.66 ± 0.27	0.632 ± 0.106	5.74 ± 0.81	8.30 ± 0.53

[a] The results are expressed as percent of injected apoprotein radioactivity present in plasma or liver at each time point after ^{125}I-labeled TRL injection. The total injected radioactivity was measured directly and the radioactivity present in each apoprotein fraction was calculated by multiplying the percent of radioactivity determined by SDS-column analysis times the total. The total remaining radioactivity was determined by multiplying the radioactivity in 1 ml of plasma at each time point by the plasma volume, assuming a plasma volume of 4.2% of the body weight (30). Similarly, the percent of radioactivity present in each apoprotein fraction was determined by SDS-column analysis and the radioactivity remaining in each apoprotein fraction was calculated by multiplying the percent times the total. The radioactivity distribution for each plasma sample was determined in duplicate and averaged. The liver apoprotein radioactivity was determined directly in duplicate following lipid extraction per ml of liver homogenate and the averaged results were multiplied by the total ml of liver homogenate to yield the total liver apoprotein radioactivity. The percent of radioactivity present in liver in each apoprotein fraction was determined by SDS-column analysis and total liver apoprotein radioactivity in each fraction was determined by multiplying the percent times the total liver apoprotein label. The percent of initial apoprotein radioactivity present in each apoprotein fraction in the liver at each time could then be calculated in the same way as the plasma results.

proteins declined rapidly and at 60 min less than 5% of the initial plasma apo B radioactivity was found in the liver. The appearance of hepatic apoprotein radioactivity was coupled to the disappearance of apoprotein from plasma and was probably a result of hepatic clearance of these apoproteins. The results of hepatic apoprotein analysis suggest that apo B directs hepatic removal of TRL since hepatic apo E and apo C content remained relatively constant. In addition, the favored removal of smaller apo B_l during the early phase of TRL metabolism suggests that particles containing predominently apo B_l are more rapidly taken up by the liver. It is interesting to note that maximum hepatic content of apo B was transient and occurred coincidently to the maximum apo B disappearance from plasma. At a time when apo B_h predominated in plasma, compared to apo B_l, maintaining a relatively constant concentration, there was little or no hepatic apo B radioactivity. The results of column analyses of plasma and hepatic apoprotein radioactivity 5 and 60 min after injection of ^{125}I-labeled TRL of several experiments are summarized in **Table 2.**

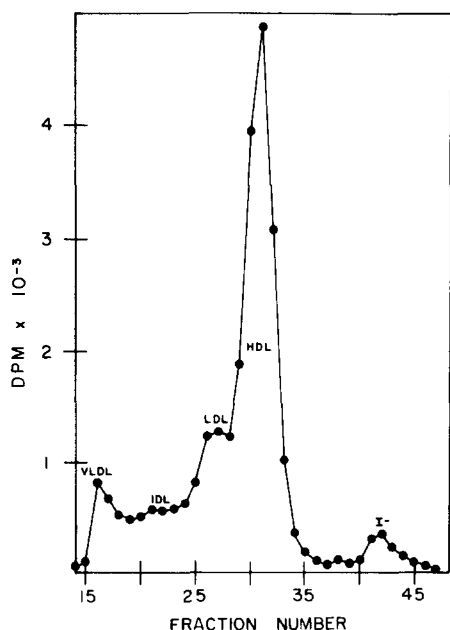

Fig. 5. Gel filtration column analysis on Sepharose CL-4B of d < 1.21 g/ml lipoproteins 30 min after injection of ^{125}I-labeled TRL. The profile of radioactivity plotted against fraction number represents the pool of four individual experiments and the lipoprotein fractions are identified as described previously (5). The ^{125}iodide peak is designated I^-. The fractions were collected at 30-min intervals at a flow rate of 7.33 ml/hr.

Apoprotein distribution among plasma lipoprotein fractions after 30 minutes

Plasma of four animals was obtained 30 min after ^{125}I-labeled TRL injection and total d < 1.21 g/ml lipoproteins were prepared by ultracentrifugation for gel filtration column analysis of lipoprotein fractions. The results are presented in **Fig. 5.** Most of the radioactivity appeared in HDL and SDS column analysis showed that almost all of this radioactivity was associated with apo C. Analysis of the d < 1.006 g/ml fraction indicated that the radioactivity again was associated entirely with apo C, probably by reassociation of apo C with freshly synthesized lipoprotein in this fraction. More than 80% of the apo B at this 30-min time was associated with LDL at a point where 90% of apo B_l had been cleared from the plasma.

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DISCUSSION

In the experiments described in this paper, plasma from rats fed a high sucrose diet was employed to prepare a triglyceride-rich d < 1.006 g/ml fraction enriched in VLDL. This TRL fraction was then labeled in vitro with ^{125}I, injected into rats, and the disappearance of individual labeled apoproteins from plasma was measured over a 1-hr period. At the same time, the appearance of labeled apoproteins in the liver was determined. In this way, the catabolism of apo B, apo E, and the C apoproteins could be followed as a function of time. The SDS-agarose column chromatographic method of analysis enabled us to separate the labeled apoproteins from whole plasma or liver homogenates without prior isolation procedures. Two labeled apo B proteins (apo B_h and apo B_l) were separated and their metabolic fates were determined.

After 5 min, only 67% of total injected apo B was found in the plasma. Almost all of this rapid loss was due to the loss of apo B_l with more than half of it removed from plasma by the liver in 5 min, while 92% of apo B_h remained in the plasma. Between 5 and 15 min, both apo B_h and apo B_l were lost rapidly from plasma. Apo B_h declined from 95% to 57% of the injected dose while apo B_l decreased from 50% to 18%. Between 15 and 60 min, there was no further decrease in plasma apo B_h; apo B_l, already low, declined further to 9% (Fig. 3). The hepatic content of apo B_h and apo B_l showed a remarkable time course (Fig. 4). Apo B_l was the dominant protein present at 5 and 15 min and both proteins reached a peak at 15 min, after which they declined precipitously so that at 60 min they were virtually absent from the liver.

It can be concluded from these experiments that apo B_h and apo B_l fractions of apolipoprotein B are heterogenous in their metabolism. We favor the concept that apo B composition is a determinant in the metabolism of TRL remnants with apo B_l favoring binding, uptake, and degradation by the liver. The TRL which was studied in these experiments was heterogeneous in that it contained both hepatic and intestine-derived TRL. In the rat, Krishnaiah, et al. (29) have found two forms of apo B using SDS-PAGE and when the apo B_h and apo B_l were isolated by SDS-columns and analyzed by SDS-PAGE they corresponded to these proteins in size. In the studies by Krishnaiah, et al. (29), the smaller protein corresponding to apo B_l was the dominant form in rat intestinal lymph. We have preliminarily extended these observations using ^{3}H-labeled amino acid incorporation into lipoproteins of intestinal mesenteric lymph and nascent hepatic lipoproteins. We conclude that apo B_l is the sole protein of intestinal lipoprotein and both apo B_l and apo B_h are synthesized by the liver.[1] The question of relative contribution of each of these proteins to the TRL plasma lipoprotein remains to be settled as does the composition of subfractions of plasma lipoprotein TRL fractions. It is evident that circulating TRL is heterogeneous in origin as well as apo B composition.

The important metabolic question is the role of apo B protein in the metabolic fate of TRL remnants. The hepatic uptake of apo B_l in preference to apo B_h does not answer this in an in vivo experiment in which heterogeneous populations of labeled TRL differing in apo B composition are injected. Apo E is known to favor and apo C to inhibit hepatic lipoprotein binding. A population of lipoproteins could exist in which the dominant apo B was apo B_l which had this favorable composition of apo B and apo C. The results are more consistent with the concept that apo B_l is the major determinant for hepatic catabolism of TRL remnants but they do not exclude a role for apo E in this process. The time course of apo B_l clearance from the plasma and appearance in the liver indicates that hepatic clearance of apo B_l from the plasma is apparently independent of apo E. In the course of TRL remnant catabolism, a constant proportion of about 10% of apo E in the plasma is present in the liver at any time point as seen in Fig. 4. In contrast, there is preferential and early clearance of apo B_l with up to 50% present in the liver by 15 min. The rapid hepatic clearance of apo B_l is best explained by binding, uptake, and degradation of TRL remnants with apo B_l as a major determinant. A role for apo E on TRL remnants containing apo B_l is not excluded by these experiments and further experiments will be necessary to determine the relative importance of apo B_l and apo E in hepatic clearance of TRL remnants.

The distribution of apo B_h and apo B_l on TRL particles in the plasma is not known. From these studies, TRL containing predominantly apo B_l which would be characteristic of intestinal TRL would be hepatically bound and degraded most rapidly. It is likely that the initial very rapid hepatic clearance of apo B_l in the first 5 min following injection (t 1/2 less than 5 min) is predominantly an indication of catabolism of intestine-derived TRL containing purely apo B_l. There is a rapid initial decay phase of apo B_h from 5–15 min (t 1/2, 15–20 min) along with a similar decay of apo B_l from 5–15 min and this phase may reflect clearance

[1] Sparks, C. E., O. Hnatiuk, and J. B. Marsh. Unpublished observations.

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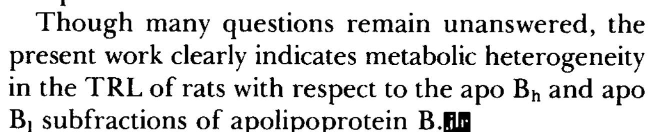
of a particle containing both apo B_h and apo B_l. The slow phase of apo B_h decay (t 1/2 greater than 2 hr) at a time when most of apo B_h is in LDL is an indication of decreased hepatic clearance. The analyses of liver homogenates at 5 min is consistent with the hepatic content of apo B_l at a ratio of apo B_l/apo B_h of 3.62 compared to 2.09 in the initial TRL.

The distribution of apo B_l and apo B_h among lipoprotein fractions and the effect of this distribution on lipoprotein metabolism is under current study. The potential exists for three types of particle composition with respect to apo B within the TRL: particles containing only apo B_l, only apo B_h, or a spectrum of particles with mixtures of apo B_l and apo B_h. A simple explanation for the data is that composition with respect to apo B_l determines the rapidity of hepatic clearance. Thus the purely apo B_l-containing TRL would be cleared most rapidly, the purely apo B_h-containing TRL would not be hepatically cleared, and the particles with mixed composition would be cleared at an intermediate rate. To prove this it would be necessary to isolate homogeneous populations of particles containing these apo B compositions and study their catabolic fate. The precursor–product relationship of VLDL to LDL is clear in humans but in the rat much of the VLDL is catabolized without conversion to plasma LDL (21). The present demonstration of the metabolic heterogeneity of apo B could provide an explanation of species differences in VLDL catabolism. Humans could possibly have more TRL containing apo B_h which preferentially enters the LDL pathway.

The recent experiments of Shelbume, et al. (11) indicate that apo C-III on the surface of chylomicrons inhibits hepatic uptake and similar observations have been made for apo C by Windler, Chao, and Havel (12). Thus, peripheral lipoprotein lipase activity may precede hepatic recognition. We propose the following hypothesis concerning the sequence of events following secretion of TRL from the liver and intestine. After reaching the circulation, peripheral lipoprotein lipase acts on TRL with shrinkage of particle size and release of surface components into HDL. The TRL remnant then binds to the liver where further lipolysis occurs by hepatic lipase action with release of surface components to HDL prior to internalization and degradation. The uptake and degradation of the TRL remnant is favored by an increased content of apo B_l. At least some of apo B_h of the TRL remnant is taken up and degraded but the remainder enters the LDL plasma pool. The role for hepatic lipase in LDL formation remains unproven but Havel, et al. (22) have suggested that remnants of VLDL are converted to LDL following hepatic binding. Alternatively, TRL containing predominantly apo B_h could be converted to LDL in peripheral tissues independently of hepatic action. A particle containing apo B_h may be bound and degraded by the liver initially but following entry of apo B_h into LDL, liver recognition is altered. The initial rapid catabolism of apo B_h could relate to its presence on a particle containing apo B_l that is hepatically catabolized. Diminished hepatic clearance of apo B_h present on LDL could relate to absence of hepatic recognition of apo B_h or possibly differences between TRL remnants and LDL either in the lipid or protein components.



Though many questions remain unanswered, the present work clearly indicates metabolic heterogeneity in the TRL of rats with respect to the apo B_h and apo B_l subfractions of apolipoprotein B.

We wish to thank Arline Ritz, Bruce LaMont, Sarah Massey, and Oleh Hnatiuk for their excellent technical assistance. This work was supported in part by N.I.H. grant No. PO1-HL-22633.

Manuscript received 19 September 1980 and in revised form 8 December 1980.

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