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Metabolism of Apolipoprotein B-100 in Large Very Low Density Lipoproteins of Blood Plasma
Kinetic Studies in Normal and Watanabe Heritable Hyperlipidemic Rabbits

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Abstract

The metabolism of radioiodinated apo B-100 in large VLDL from normal and Watanabe heritable hyperlipidemic (WHHL) rabbits, with diameters exceeding 450 Å, was studied in corresponding recipient rabbits. In both cases ~ 87% of the particles contained apolipoprotein (apo) E (B,E particles). In normal rabbits, apo B in these B,E particles was removed from blood plasma much more rapidly than apo B in B,E particles in smaller VLDL and few of the large B,E particles were converted to lipoproteins of higher density. In WHHL rabbits, ~ 60% of the apo B in B,E particles in large VLDL was removed at a comparably rapid rate, but an appreciable fraction of the remainder, which was removed slowly, was converted to particles of higher density, as are the B,E particles in smaller VLDL. From kinetic analysis of these and other data, an hypothesis was formulated from which it is estimated that apo B in large VLDL accounts for 18 and 41% of apo B transport in normal and WHHL rabbits, respectively, despite the fact that these lipoproteins contain < 5% of the apo B in total VLDL. Failure to account for the contribution of large VLDL to VLDL turnover may lead to serious underestimation of total apo B transport in the blood.

Introduction

Recent research has suggested that the presence of apo E on VLDL particles in rabbit blood plasma has a major influence on their metabolism (1, 2). In normal New Zealand white (NZW)[1] rabbits, most VLDL particles contain apo E as well as apo B-100 (B,E particles), but others lack apo E (B particles). B,E particles in VLDL are removed from the blood much more rapidly than B particles and a smaller fraction of B,E particles is converted to particles of higher density, including intermediate density lipoproteins (IDL) and LDL. We have proposed that the presence of more than one molecule of apo E on VLDL particles enhances their binding to LDL receptors in the liver, promoting their removal from the blood and limiting their conversion to IDL and LDL.

In Watanabe heritable hyperlipidemic (WHHL) rabbits, which are grossly deficient (but perhaps not totally lacking) in functional LDL receptors, VLDL accumulate in the blood as a result of impaired hepatic uptake and a larger fraction is converted to IDL and LDL than in normal rabbits, consistent with a key role for the LDL receptor in the direct removal of partially metabolized VLDL (VLDL remnants) from the blood (2, 3). However, as in normal rabbits, B,E particles in VLDL of WHHL rabbits are removed directly from the blood much more efficiently than B particles (2). This observation led us to propose that some VLDL particles (possibly those that contain several molecules of apo E) are removed from the blood of WHHL rabbits by interaction with a lipoprotein receptor, either the chylomicron remnant receptor or the structurally abnormal LDL receptor that is known to be synthesized in WHHL rabbits (2, 4). Although the mutant receptor in WHHL rabbits is poorly glycosylated, a fraction may be transported to the plasma membrane of cells. In humans, large VLDL particles are removed from the blood much more rapidly than smaller ones and few large VLDL are converted to lipoproteins of higher density (5, 6). In the current research, we have therefore tested the hypothesis that large VLDL make a substantial contribution to the transport of particles containing apo B into the blood of both normal and receptor-deficient rabbits. Our results provide support for this hypothesis, despite the fact that large VLDL account for only a small fraction of the VLDL present in blood plasma.

Methods

Animals. Male NZW rabbits weighing 2.2–2.8 kg and WHHL rabbits weighing 2.1–2.7 kg were fed Purina Lab Rabbit chow (Ralston Purina Co., St. Louis, MO). Before and during in vivo experiments, the animals were kept in individual cages (1). The mean plasma total cholesterol and triglyceride concentrations were 33 and 81 mg/dl in NZW rabbits; corresponding values in WHHL rabbits were 496 and 373 mg/dl.

Preparation of lipoproteins. Blood was withdrawn from a femoral vein of tranquilized donor rabbits (1) and mixed with disodium EDTA (1 mg/ml), sodium azide (0.2 mg/ml), and gentamycin (10 μg/ml), pH 7.4 (buffer A). Fresh plasma, obtained at 4°C, was centrifuged in a Beckman SW 41 rotor (Beckman Instruments, Inc., Fullerton, CA) at 10,000 rpm for 30 min at 12°C to minimize contamination of VLDL with particles containing apo B-48; the top 1 ml of the tube contents was removed by tube slicing. "Large" VLDL were then isolated from the remaining plasma in an SW 41 rotor at 39,000 rpm for 1 h at 12°C; the top 1 ml was obtained by tube slicing. This lipoprotein fraction was layered beneath 1.5 ml 0.075 M NaCl and centrifuged twice under the same conditions, dialyzed against 0.15 M NaCl containing 0.01% disodium EDTA, 0.02% sodium azide, pH 7.4, and iodinated with [125]I (Amersham Corp., Arlington Heights, IL) by a modification of the method of McFarlane (7) to a specific activity of 50–250 dpm/ng protein. Free iodine was removed on a column (1 × 10 cm) of Sephadex G-50 (Pharmacia Fine Chemicals, Piscataway, NJ). As determined

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1. *Abbreviations used in this paper:* NZW, New Zealand White (rabbits); WHHL, Watanabe heritable hyperlipidemic (rabbits).

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by assay of components separated by SDS gel electrophoresis (5) from two preparations from NZW rabbits and one preparation from WHHL rabbits, 90–92% of the ^{125}I-apo B in large VLDL from both NZW and WHHL rabbits was in apo B-100 and 8–10% in apo B-48. The percentage of total radioiodine in apo B, measured as ^{125}I insoluble in 50% isopropanol, was $\sim$ 29 in large VLDL from NZW rabbits and 15 in that from WHHL rabbits. The labeled lipoproteins (0.5–1.0 mg protein) were mixed with 1 ml of donor plasma and dialyzed twice against 4 liters of buffer A at 4°C. Before injection into animals, the iodinated lipoproteins were dialyzed against 0.15 M NaCl for 1–2 h and passed through a 0.22-μm filter (Gelman Sciences, Inc., Ann Arbor, MI). In some experiments, VLDL were separated from fresh plasma on a 2 × 90-cm column of 4% agarose gel (Bio-Gel A-15 M; Bio-Rad Laboratories, Richmond, CA) (8).

Lipoprotein kinetics in vivo. Large VLDL (30–60 μCi) were injected into an ear vein of unanesthetized animals at $\sim$ 9 a.m. Blood samples of 2 ml were taken from an opposite ear vein into tubes containing 2 mg disodium EDTA and 0.4 mg sodium azide 3, 30, 90, 180, 360, 720, and 1,440 min after injection and placed on ice. Plasma was separated by centrifugation at 4°C.

Immunoaffinity chromatography and density gradient ultracentrifugation. B and B,E particles were separated from 0.02 to 0.10 ml whole rabbit plasma on columns of affinity-purified sheep antirabbit apo E coupled to Sepharose 4B (2). The unbound fraction of plasma applied to the column contained no detectable apo E by RIA (9). Samples of plasma were also subjected to density gradient ultracentrifugation and 12 fractions with densities ranging from 1.005 to 1.050 g/ml were obtained as described (2).

Measurement of radioiodine in apo B. Radioiodine in apo B of whole plasma and in the fraction that did not bind to the immunoaffinity column (B particles) was assayed as described (10). Radioiodine in apo B of lipoproteins bound to the column (B,E particles) was determined by subtracting radioactivity in apo B of the unbound fraction from that of apo B in whole plasma.

To determine the fraction of labeled apo B in total VLDL that is accounted for by large VLDL, total VLDL from each of five NZW rabbits were radioiodinated. The labeled VLDL were centrifuged as described above for separation of large VLDL, and portions of the supernatant (which contained large VLDL together with some contaminating smaller VLDL particles) and the infranatant were taken for assay of ^{125}I-apo B. The remainder of the supernatant was recentrifuged under the same conditions and the infranatant was taken for assay of ^{125}I-apo B. Apo B radioactivity in large VLDL was calculated from the difference between that in the supernatant from the first centrifugation and the infranatant from the second centrifugation. Apo B radioactivity in the remainder of the VLDL particles was calculated as the sum of that in the infranatants from the two centrifugations.

Lipoprotein analysis. Total cholesterol and triglycerides were measured in plasma by an automated chemical method (11). Total cholesterol, free cholesterol, and triglycerides in lipoprotein fractions were measured by enzymatic methods (12, 13). Phospholipids were estimated from content of lipid phosphorus (14), and protein was measured by a modified Lowry procedure (15). Apo B was estimated as isopropanol-insoluble protein (1). Electron microscopy of lipoproteins was performed on samples negatively stained with potassium phosphotungstate (16). Particle diameters were measured on the photographic prints at a magnification of 180,000 (9).

Kinetic analysis. The concentration of ^{125}I-apo B in B,E and B particles in whole plasma was measured in six NZW rabbits and four WHHL rabbits after injection of large VLDL. Inspection of the data revealed that a significant portion of B,E and B particles in large VLDL of NZW rabbits and of B,E particles in large VLDL of WHHL rabbits have residence times considerably shorter than those seen when total VLDL are injected (2). These new data, averaged for NZW and WHHL rabbits, together with previously published data on B,E and B particle kinetics after injection of total VLDL (2), were used to construct a mechanistic hypothesis describing the kinetics of large, rapidly

turning-over VLDL particles in NZW and WHHL rabbits. Our analysis indicated that in NZW rabbits, most but not all of the B,E and B particles in large VLDL turn over rapidly. In the following, we will refer to these rapidly turning-over over particles as "fast B,E" and "fast B" particles. The remaining particles in large VLDL, like the great majority of those in total VLDL, will be referred to as "slow B,E" and "slow B" particles. The fast B,E and B particles are not clearly evident upon inspection of the disappearance data in whole plasma when total VLDL are injected because they constitute only a small fraction of total VLDL particle number (see below). A similar situation was found to exist in WHHL rabbits.

The modeling process was performed with the SAAM 27 computer program (17, 18). The following hypotheses were tested. (*a*) Fast B,E and B particles in both NZW and WHHL rabbits are delivered to the plasma and exit the plasma without conversion to slow VLDL particles, IDL, or LDL. (*b*) The residence times of fast B,E particles in NZW and WHHL rabbits are equal and very short as compared with those of slow B,E particles. (*c*) The fractional rates of irreversible loss of fast and slow B particles from the plasma of NZW and WHHL rabbits are equal to the corresponding fractional rates of irreversible loss of LDL particles from plasma. (*d*) Fast B particles in NZW and WHHL rabbits are partially converted to fast B,E particles. (*e*) The fractional conversion of slow B,E and B particles in VLDL to B,E and B particles in IDL is equal but different in NZW and WHHL rabbits. (*f*) All apo B enters the plasma as VLDL (19).

The percentage of whole VLDL in NZW rabbits represented by large particles was determined experimentally and contains a high proportion of fast B,E and B particles, together with some slow B,E and B particles (see Results). This differs from total VLDL, in which nearly all the B,E and B particles are slow. Therefore, although both sets of tracer data (after injection of large and total VLDL) in NZW and WHHL rabbits contain both fast and slow B,E and B particles, the presence of the fast particles is much more obvious after injection of large VLDL. IDL and LDL subsystem kinetics in NZW and WHHL rabbits are assumed known from our previous data and analysis (1, 2). As in our previous work (1, 2), the whole plasma tracer data for NZW and WHHL rabbits are analyzed as combinations of radiolabeled apo B in fast VLDL, slow VLDL, IDL, and LDL in either B,E or B particles.

Given our new data on large VLDL together with our previous analyses for total VLDL, IDL, and LDL and applying the hypotheses stated above, the rate constants for only four pathways are adjustable in the model solution for NZW rabbits and for three pathways in the model solution for WHHL rabbits. In Fig. 4 and 5, these pathways are indicated by heavy arrows. In NZW rabbits, the four rate constants include (*a*) the fractional rate of irreversible loss of fast VLDL B,E particles from plasma; (*b*) the fractional rate of irreversible loss of slow VLDL B,E particles from plasma; (*c*) the fractional rate of conversion of fast B particles to fast B,E particles in VLDL; (*d*) the fractional rate of conversion of slow VLDL B,E particles to IDL B,E particles. The three adjustable rate constants for WHHL rabbits include all but the first of these.

The model requires 16 initial conditions (four for each injection of large and total VLDL in NZW and WHHL rabbits) to be established from eight sets of tracer data (plasma ^{125}I-apo B in B,E and B particles after large and total VLDL injections in NZW and WHHL rabbits). This was accomplished with the adjustable initial condition feature of the SAAM 27 program after various constraints were applied to provide uniqueness (see Appendix). The concentrations of apo B in fast and slow B,E and B particles in total VLDL of NZW and WHHL rabbits were calculated from the measured B,E and B particle concentrations of apo B in total VLDL (2) and from the relative concentrations of ^{125}I-apo B in fast and slow B,E and B particles in total VLDL. The latter were determined from the estimated initial conditions of fast and slow B,E and B particles in radiolabeled total VLDL of NZW and WHHL rabbits. The details of these calculations, which are based upon the tracer data and measurement of the fraction of apo B in total VLDL that is large, are given in the Appendix. Using these calculated

values for the concentrations of fast and slow B,E and B particles in VLDL, a steady state solution was obtained for both NZW and WHHL rabbits, yielding the transport rates of apo B in VLDL, as well as in IDL and LDL. The measured steady state concentrations of apo B in B,E and B particles in IDL and LDL in NZW and WHHL rabbits (2) were weighted in the data set and served to constrain the model fits to the tracer data. All adjustable parameters and initial conditions were fitted to the eight sets of tracer data for apo B in whole plasma at the same time using the multiple study capability of the SAAM 27 program.

The uncertainties of the adjustable model parameters and the adjustable initial conditions were estimated from the variance-covariance matrix in the neighborhood of the least-squares fit. The details of the fitting procedure have been presented elsewhere (1, 2).

Results

Large VLDL from NZW and WHHL rabbits were of similar size, with mean diameters, estimated from their composition (20), of 641 and 702 Å, respectively. As with total VLDL, large VLDL from WHHL rabbits contained more cholesteryl esters and less triglycerides than those from NZW rabbits (Table I). The distributions of particle size in electron micrographs of negatively stained preparations of large VLDL from NZW and WHHL rabbits were similar (Fig. 1). More than 80% of particle diameters were between 500 and 900 Å, whereas particle diameters of > 90% of total VLDL were below 500 Å. By direct measurement, apo B in large VLDL of NZW rabbits comprised 5.26% ($n = 5$, SEM = 0.90) of that in total VLDL. The number of apo E molecules per particle of large VLDL (which had been subjected to ultracentrifugation three times) was less than two, as estimated from the molar ratio of apo E to apo B. In VLDL fractions of whole plasma eluted from 4% agarose gel columns, the E:B ratio increased progressively with particle size, from 1.30 to 2.63 in NZW rabbits and from 1.18 to 2.52 in WHHL rabbits. However, the concentration of apo E in the largest particles, which represented 12 and 6% of the mass of apo B in VLDL from NZW and WHHL rabbits, respectively, was below the limit of sensitivity of the apo E RIA. About 87% of ^{125}I-apo B in large VLDL from both NZW and WHHL rabbits was in B,E particles (Table II). Similar values were obtained for total VLDL from WHHL rabbits, whereas a lower value (75%) was found for total VLDL from NZW rabbits.
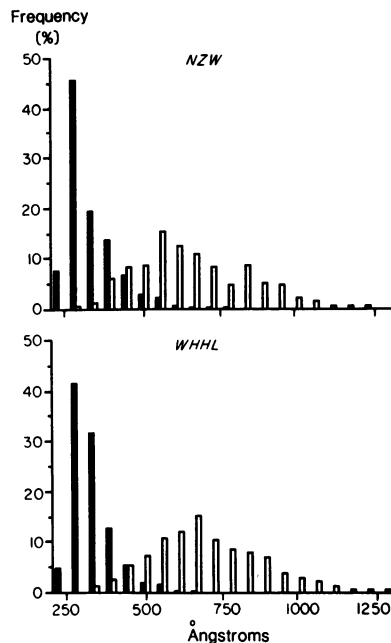

Figure 1. Distribution of particle diameters measured on photographic prints of negatively stained rabbit VLDL. (Closed columns) Total VLDL, mean values for four samples from NZW rabbits and two samples from WHHL rabbits. (Open columns) Large VLDL, mean values for two samples each from NZW and WHHL rabbits.

The kinetics of removal of radioiodine in total apo B from blood plasma after injection of labeled large VLDL into NZW and WHHL rabbits are shown in Fig. 2 and compared with those observed after injection of total VLDL. In both groups of rabbits, ^{125}I in apo B of large VLDL was removed from plasma much more rapidly than that of total VLDL. In NZW rabbits, 75% was removed during the first 30 min and < 1% remained after 12 h. In WHHL rabbits, almost 50% was removed during the first 30 min, but thereafter the rate of removal slowed markedly and resembled that of apo B in total VLDL. In all cases, ^{125}I-apo B in B,E particles was removed more rapidly than that in B particles (Fig. 3). In NZW rabbits, both B,E and B particles in large VLDL were removed more rapidly than their counterparts in total VLDL. In WHHL rabbits, B,E particles in large VLDL were initially removed more rapidly than those in total VLDL. As these figures demonstrate, the model solutions fit the tracer data well.

The fractional turnover rates (h^{-1}), transport rates ($mg \cdot dl^{-1} \cdot h^{-1}$) and calculated concentration ($mg \cdot dl^{-1}$), based on both sets of injections into NZW rabbits, are shown with the model in Fig. 4 and those for WHHL rabbits are shown in Fig. 5. All adjustable parameters and initial conditions were well determined with maximum fractional standard deviation (FSD) of 0.19 and average FSD of 0.06. The calculated initial distributions of ^{125}I-apo B for total VLDL and large VLDL injections into NZW and WHHL rabbits, expressed as percent

Table I. Composition of Plasma VLDL

	NZW		WHHL	
	Total ($n = 6$)	Large ($n = 7$)	Total ($n = 6$)	Large ($n = 3$)
	% mass			
Cholesteryl ester	5.7±1.3	3.4±1.2	26.5±5.8	14.7±13.0
Triglyceride	58.7±4.2	74.6±4.4	39.4±0.8	65.6±16.9
Cholesterol	5.7±1.0	3.3±0.7	5.8±0.6	3.9±2.2
Phospholipid	18.9±2.1	12.9±1.5	17.4±5.4	12.0±2.0
Protein	11.2±1.6	5.7±1.9	10.1±1.5	3.9±0.7
Diameter (Å)*	367	641	390	702
Molar ratio: E/B‡	2.09	1.65	2.40	1.86

* Calculated from composition (20).
‡ Based on M_r for apo B of 512,000 and for apo E of 34,000.

Table II. Distribution of ^{125}I-apo B between B and B,E Particles in Plasma VLDL

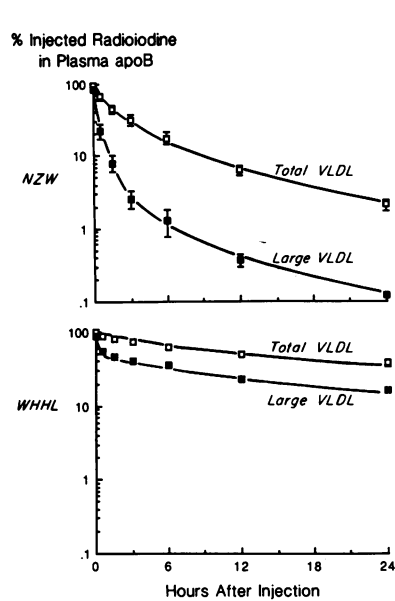|	Total VLDL		Large VLDL	
	NZW	WHHL	NZW	WHHL
B,E particles	75.2±4.4	87.7±3.9	87.7±2.7	85.6±4.1
B particles	24.8±4.3	12.3±3.8	12.3±2.3	14.4±3.5

Values are mean percentages±SD for four to six samples.

Figure 2. Removal of ^{125}I-apo B from whole blood plasma after injection of homologous total VLDL or large VLDL into NZW and WHHL rabbits. Mean values for total VLDL are from references 1 and 2. Mean values for large VLDL are for six experiments in NZW rabbits and four experiments in WHHL rabbits. Bars represent 1 SE (where not shown, SE values fall within data points). The curves were derived from kinetic analysis of the data using the model shown in Figs. 4 and 5.

Figure 4. Multicompartmental model of the metabolism of apo B-100 in NZW rabbits. Solution from analysis of data on B,E and B particles in whole plasma after injection of large VLDL, total VLDL, IDL, and LDL. The concentrations (mg/dl) of B,E and B particles of lipoprotein classes in the intravascular compartment are shown within the circles. Empty circles represent extravascular compartments for IDL and LDL. Transport rates (mg · dl⁻¹ · h⁻¹) are underlined. Small, italicized numbers are rate constants (h⁻¹). Adjustable rate constants are shown with heavy arrows. VLDL include fast and slow components for both B,E and B particles; the fast components, which represent ∼ 95% of large VLDL injected in the current study, represent only ∼ 5% of VLDL apo B mass (see Table III).

of total radioactivity in VLDL apo B, are shown in Table III. The percent of radiolabeled apo B in fast B,E and B particles in total VLDL can be calculated to be 5.0 and 3.3% in NZW and WHHL rabbits, respectively. Assuming that apo B labeling is proportional to apo B mass (2), these percentages also reflect the relative concentrations of apo B in fast B,E and B particles in these rabbits, as shown in Figs. 4 and 5.

The total apo B production rates in NZW and WHHL rabbits are calculated to be 9.53 and 8.23 mg·dl⁻¹·h⁻¹, respectively. The fractions of the apo B production rates represented by fast particles are 18 and 41% in NZW and WHHL rabbits, respectively. The fractions represented by B,E particles (fast and slow) are 92 and 95%, respectively. The discrepancy between production rate and mass is particularly large for fast B,E particles in WHHL rabbits for which the fractional irreversible disposal rate exceeds that of slow B,E particles by
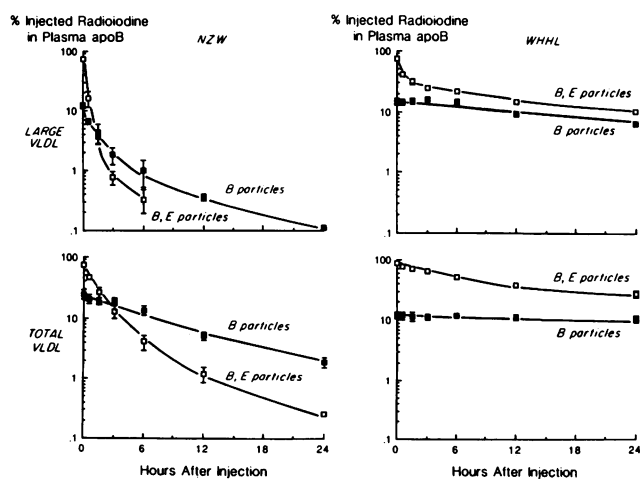
∼ 50-fold. These fast B,E particles account for 41% of total apo B transport, but only 2.8% of apo B mass (Fig. 5 and Table III, respectively).

Figure 3. Removal from whole blood plasma of ^{125}I-apo B in B,E and B particles after injection of homologous radioiodinated lipoproteins into rabbits. Data are from the same experiments shown in Fig. 2. Bars represent 1 SE (where not shown, SE values fall within data points). The curves were derived from kinetic analysis of the data using the model shown in Figs. 4 and 5.
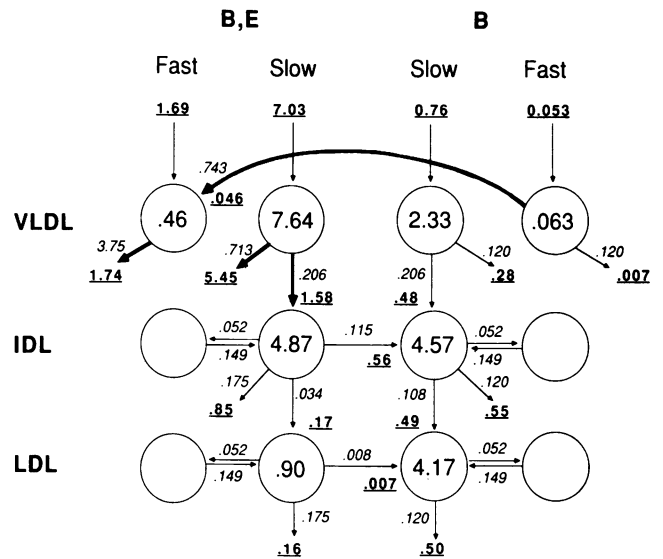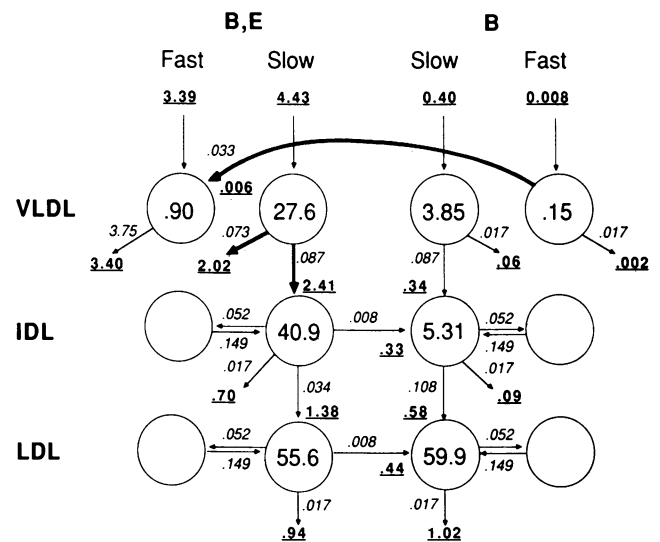

Figure 5. Multicompartmental model of the metabolism of apo B-100 in WHHL rabbits: solution from analysis of the data on B,E and B particles in whole plasma after injection of large VLDL, total VLDL, IDL, and LDL. Circles, arrows, and numbers are represented as in Fig. 4. VLDL include fast and slow components for both B,E and B particles; the fast components, which represent ∼ 62% of large VLDL injected in the current study, represent only ∼ 3% of VLDL apo B mass (see Table III).

*Table III. Calculated Initial Distributions of
^{125}I-Apo B in Kinetically Fast and Slow B,E and B Particles
after Injection of Total and Large VLDL*

| | Percent ^{125}I-apo B | | | |
| | Total VLDL | | Large VLDL | |
	NZW	WHHL	NZW	WHHL
B,E particles				
Fast	4.4	2.8	83.8	52.9
Slow	72.8	84.9	3.9	32.7
B particles				
Fast	0.6	0.5	11.1	8.9
Slow	22.2	11.8	1.2	5.5

The model assumption that fast VLDL particles are not converted to IDL and LDL is supported by analyses of the distribution of ^{125}I-apo B in lipoprotein fractions obtained after density gradient ultracentrifugation of the samples taken up to 24 h after injection of large VLDL. In NZW rabbits, ^{125}I rose to ~ 8% of injected ^{125}I-apo B in lipoproteins with densities > 1.006 g/ml 30 min after injection, but quickly fell to undetectable levels (< 0.5%). These small amounts of ^{125}I-apo B in IDL + LDL probably reflect incomplete flotation of VLDL. In WHHL rabbits, very little ^{125}I-apo B appeared in lipoproteins denser than 1.006 g/ml during the rapid phase of removal (up to 30 min after injection). Thereafter, ^{125}I-apo B appeared gradually in lipoproteins of progressively increasing density up to 1.04 g/ml, accounting for 8% of injected ^{125}I-apo B 6 h after injection (data not shown). This indicates that some of the apo B in the injected large VLDL of WHHL rabbits, with kinetic behavior similar to that of the majority of VLDL particles, was converted to IDL and LDL.

Discussion

In our earlier studies of the metabolism of apo B-100 in WHHL rabbits (2), we observed that the fractional rate of irreversible loss of VLDL B,E particles from plasma considerably exceeded that of B,E particles in IDL or LDL, as well as those of B particles in all density classes of lipoproteins containing this apoprotein. A plausible explanation of this observation is that some VLDL B,E particles are recognized by a lipoprotein receptor in these LDL receptor-deficient animals. Since we had observed that large VLDL (with diameters exceeding 470 Å) are removed from the blood of humans much more rapidly than the majority of VLDL particles and at rates approaching those expected for chylomicrons (5), we have evaluated the metabolism of similarly large VLDL in blood plasma of NZW as well as WHHL rabbits. In both groups of rabbits, only a small fraction of VLDL particles has diameters > 500 Å. Hence, to prepare VLDL of this size it was necessary to subject them to repeated flotation to reduce to low levels particles < 500 Å. Most of the large VLDL obtained from both groups of rabbits were > 90% of total VLDL and most of them contained apo E. However, the number of apo E molecules found per particle was less than that expected from previous observations of human VLDL (21). Since the lipoproteins had been ultracentrifuged three times, an appreciable fraction of

the apo E may have been dissociated. This possibility is consistent with the ratios found in subfractions of VLDL separated from whole plasma by gel chromatography. For this reason, in the current experiments, as in those reported previously, we reincubated the radiolabeled lipoproteins with plasma of the recipient animals before injection to permit them to regain apo E.

The large VLDL we isolated account for only a small fraction of VLDL particles. Their fractional rate of irreversible loss from plasma would therefore have to exceed that of the remainder of VLDL by severalfold for them to have an appreciable influence on the metabolism of total VLDL. Based on the kinetic hypothesis presented here, we found this to be the case in NZW rabbits, as in humans (5). The estimated fractional rate of irreversible loss of fast B,E particles in large VLDL from plasma was fivefold greater than that of slow B,E particles. In addition, consistent with previous observations of the behavior of large human VLDL (5, 6), few of the large VLDL particles were converted to particles more dense than 1.006 g/ml.

In WHHL rabbits, the fractional rate of irreversible loss of ~ 60% of the B,E particles in large VLDL from plasma exceeded that of B,E particles in smaller VLDL by a factor of 50. Indeed, it was possible to constrain this fractional rate to be the same as that observed for NZW rabbits, consistent with the hypothesis that some VLDL B,E particles in WHHL rabbits are metabolized normally. In this connection, three points warrant emphasis. First, only ~ 9% of the labeled apo B of large VLDL in WHHL rabbits was in apo B-48, but more than one-half of the apo B in large VLDL was metabolized rapidly. It follows that at least 80% of the apo B in rapidly metabolized large VLDL was in apo B-100. Second, ~ 40% of the apo B in B,E particles of large VLDL (slow B,E particles) seems to be removed from the blood with kinetics similar to those seen when total VLDL are injected (i.e., ~ 50-fold more slowly). Third, the average fractional rate of irreversible loss from plasma of slow B,E particles in VLDL was calculated to be 0.071 h^{-1} (Fig. 5), a value about fivefold greater than that of apo B in all other lipoprotein fractions. Such kinetic heterogeneity of VLDL in these receptor-deficient animals could be related to variable content of apo E molecules or to variability in the conformation of the receptor-binding domain of apo E.

Based upon the model presented above, the total apo B production rate in WHHL rabbits is ~ 86% of that calculated for NZW rabbits. This value compares favorably with the relative rates of accumulation of apo B in perfusates of isolated livers from NZW and WHHL rabbits. The production rate of apo B in WHHL rabbits was ~ 80% of that found in NZW rabbits (19).

The disappearance of ^{125}I in B particles from plasma after injection of large VLDL into NZW and WHHL rabbits clearly reveals a component that turns over more rapidly than that of B particles after injection of total VLDL (Fig. 3). To explain this phenomenon we have postulated that some fast B particles are converted to fast B,E particles. The fractional rate of irreversible loss of B particles from plasma was assumed to be equal to that of LDL B particles. Two other hypotheses, equally consistent with the tracer data, are those of complete rather than partial conversion of fast B particles to fast B,E particles and of a fractional irreversible rate of loss of fast B particles from plasma greater than that of LDL B particles. In both of these cases, the main conclusions drawn from our analysis would not be materially affected because less than 1%

of the total apo B transport occurs through this fast B particle pathway (Figs. 4 and 5).

The model solutions shown in Figs. 4 and 5 explain the early rapid disappearance of B,E particles in large VLDL by irreversible removal from plasma. We have evaluated several other hypotheses that might explain this phenomenon. The first is based upon the fact that triglyceride-rich lipoproteins initially bind to lipoprotein lipase at the surface of capillary endothelia. Particles that have a high affinity for the enzyme might thereby leave the plasma rapidly, but transiently (22). To test this hypothesis, we included in the model shown in Figs. 4 and 5 an extravascular compartment that equilibrates with the pool of fast B,E particles in plasma, and assumed that there are no slow B,E particles in large VLDL. For NZW rabbits, results consistent with the tracer data were obtained, but only with a mass of apo B in the extravascular compartment that exceeded that in plasma by a factor of $\sim$ 300. Since such a massive compartment seems very unlikely, we have rejected this hypothesis for NZW rabbits. For WHHL rabbits, in which a substantial fraction of apo B in large VLDL remained in plasma for a prolonged period of time, results consistent with the tracer data were also obtained. In this case, the mass of apo B in the extravascular compartment exceeded that in fast B,E particles in plasma by a factor of about three. Under these conditions, the production rate of fast B,E particles is only 0.20 mg $\cdot$ dl^{-1} $\cdot$ h^{-1}, as compared with the value of 3.39 mg $\cdot$ dl^{-1} $\cdot$ h^{-1} shown in Fig. 5. The fractional rate of movement of fast B,E particles into the extravascular pool is about 2.2 $\cdot$ h^{-1}, and the fractional rate of irreversible loss of fast B,E particles from plasma is 0.14 $\cdot$ h^{-1}, about double that for slow B,E particles in VLDL. This hypothesis fails to explain the observation that B,E particles in large VLDL that persisted in the plasma of WHHL rabbits were converted appreciably to IDL and LDL. Furthermore, this hypothesis is not consistent with the relatively large size of the VLDL particles in isolated liver perfusates from WHHL rabbits (19). Therefore, a second alternative hypothesis was tested for WHHL rabbits, namely that fast B,E particles in large VLDL rapidly bind to lipoprotein lipase sites on capillary endothelia and then return quantitatively to the plasma as VLDL remnants (contained within slow B,E particles), which are removed only slowly from plasma, with gradual conversion to IDL and LDL. It was not possible to fit this model to the tracer data, since the return of remnant particles to the plasma yielded a substantial increase in tracer shortly after its initial removal.

A third alternative hypothesis, which combines an extravascular compartment equilibrating with fast B,E particles in plasma with some conversion of extravascular B,E particles to slow B,E particles in plasma was consistent with the data of Fig. 3. However, like the first alternative hypothesis discussed above, this one is not consistent with the rate of appearance of apo B in IDL and LDL in WHHL rabbits during the first 6 h after injection of large VLDL.

Finally, a fourth alternative hypothesis was tested that explains the kinetics of the disappearance of B,E and B particles from plasma after injection of large VLDL by partial conversion of fast B,E and B particles to slow B,E and B particles, respectively. This hypothesis is overtly consistent with the concept of a delipidation cascade in VLDL which is included within the VLDL compartments of Figs. 4 and 5. It assumes that all of the apo B in large VLDL is contained in fast B,E and B particles. This model is consistent with all of the data shown

in Fig. 3 as well as the data from density gradient ultracentrifugation. The transport rates for NZW and WHHL rabbits calculated in the steady state solution of this model are generally similar to those shown in Figs. 4 and 5, with the exception of the production rate of fast B,E particles in WHHL rabbits. This rate, 5.33 mg $\cdot$ dl^{-1} $\cdot$ h^{-1}, is 65% of the total apo B production rate in these animals and would require a very large average size of nascent lipoprotein particles. Given the data currently available, we cannot favor our initial hypothesis over this alternative. Nevertheless, no major differences other than the above exist between these two hypotheses.

The implications of our kinetic analysis are consistent with our earlier suggestion that some particles containing apo B-100 are removed from the blood by WHHL rabbits via interaction with a lipoprotein receptor. Whether fast B,E particles are removed by chylomicron remnant receptors or by structurally abnormal LDL receptors that reach the surface of hepatocytes remains uncertain. The fact that the fractional irreversible disposal rates for fast B,E particles could be constrained to be equal in NZW and WHHL rabbits is, however, consistent with removal via a chylomicron remnant receptor.

The observations and analysis reported here are critical to an understanding of the metabolism of particles containing apo B-100 in WHHL rabbits. They suggest that $\sim$ 40% of the secreted lipoprotein particles containing this protein are metabolized rapidly and at an apparently normal rate in these receptor-deficient animals. They lead to the prediction that the secreted VLDL are considerably larger than those isolated from plasma. The diameter of $\sim$ 40% of secreted VLDL should exceed 500 Å, whereas only 3% of particles in plasma VLDL are this large. Similar predictions apply to VLDL in NZW rabbits. About 18% of secreted VLDL particles should be larger than 500 Å in diameter, compared with only about 5% of those in blood plasma. These implications of our model, that the size of secreted particles containing apo B is considerably larger than that found in plasma and that this size is greater in WHHL rabbits than in NZW rabbits, are consistent with the observations of Hornick and associates on the size of VLDL that accumulate in perfusates of isolated rabbit livers (19). From their data on the composition of these VLDL, the mean diameters of particles from liver perfusates from NZW and WHHL rabbits weighing $\sim$ 1.5 kg can be estimated to be 580 and 730 Å, respectively. In addition, we have obtained preliminary data which show that VLDL isolated from Golgi apparatus-rich fractions of livers from NZW rabbits contain a substantial fraction of particles with diameters exceeding 500 Å (unpublished observations).

Our results indicate that experiments with total radioiodinated VLDL provide an incomplete picture of the metabolism of VLDL in NZW or WHHL rabbits. In both normal and receptor-deficient animals, an appreciable fraction of secreted VLDL is metabolized so rapidly that it contributes little to the mass of plasma VLDL. The contribution of these particles to the transport of VLDL cannot be appreciated from the kinetics of apo B in total plasma VLDL, given the sampling frequencies we used and the level of analytical precision that we obtained. In fact, the total apo B production rates in NZW and WHHL rabbits, based on injection of labeled total VLDL previously reported by us, are 16 and 41% lower, respectively, than those reported here. This underestimation is almost completely accounted for by failure to include transport through the fast B,E pathway in VLDL.

These observations may have important implications for the interpretation of the kinetics of plasma VLDL in other species, including humans. Previous analyses of kinetic data may underestimate the rate of entry of apo B-100 into plasma VLDL and overestimate the fraction of secreted VLDL particles that is converted to IDL and eventually to LDL. As indicated from the current studies, the magnitude of such errors may not be the same in different situations. Clearly, methods such as those described here or other approaches are needed to understand the influence of physiological and pathological conditions on the rate of secretion of apo B-100 from the liver and the fate of particles containing this important protein.

Appendix

The modeling process we have performed is based on the presence of both fast and slow B,E and B particles in large and total VLDL of NZW and WHHL rabbits. The presence of the fast B,E and B particles in plasma after total VLDL injection is poorly reflected in the kinetics of these particles due to their low concentration and the sampling frequency employed. However, the kinetic behavior of these fast particles is much better seen when their relative concentration is greatly increased by ultracentrifugal isolation of large VLDL. Analysis of the kinetics of B,E and B particles after injection of large VLDL, combined with a concurrent analysis of our data obtained after injection of total VLDL, allows estimation of the initial conditions and relative concentrations of both fast and slow B,E and B particles in total VLDL.

A set of equations describing the initial conditions for the model shown in Figs. 4 and 5, as fitted to the data of Fig. 3, can be elaborated using adjustable parameters P(I) in the SAAM 27 computer program. Let $IC(I \cdot J \cdot K)$ be the initial condition where I is either a fast (f) or slow (s) particle, J is either a B,E or B particle, and K is either an NZW (N) or WHHL (W) rabbit. The $IC(I \cdot J \cdot K)$ are expressed as percent of injected dose; the plasma concentrations at time zero are assumed to be 100%.

		Total VLDL	Large VLDL
NZW		$IC(f \cdot B,E \cdot N) = P(1)$	$IC(f \cdot B,E \cdot N) = P(5)$
		$IC(s \cdot B,E \cdot N) = P(2)$	$IC(s \cdot B,E \cdot N) = P(6)$
		$IC(f \cdot B \cdot N) = P(3)$	$IC(f \cdot B \cdot N) = P(7)$
		$IC(s \cdot B \cdot N) = P(4)$	$IC(s \cdot B \cdot N) = P(8)$
WHHL		$IC(f \cdot B,E \cdot W) = P(9)$	$IC(f \cdot B,E \cdot W) = P(13)$
		$IC(s \cdot B,E \cdot W) = P(10)$	$IC(s \cdot B,E \cdot W) = P(14)$
		$IC(f \cdot B \cdot W) = P(11)$	$IC(f \cdot B \cdot W) = P(15)$
		$IC(s \cdot B \cdot W) = P(12)$	$IC(s \cdot B \cdot W) = P(16)$

The following assumptions are applied to this set of equations allowing a unique solution subject to the requirement of fitting the tracer data shown in Fig. 3.

(*1*) Conservation of mass relations

$$\sum_{I=1}^{4} P(I) = \sum_{I=5}^{8} P(I) = \sum_{I=9}^{12} P(I) = \sum_{I=13}^{16} P(I) = 100,$$

allows P(4), P(8), P(12), and P(16) to become dependent.

(*2*) The percentage of total and large VLDL radioactivity constituted by B,E particles as shown in Table 2 is used to make P(2), P(6), P(10), and P(14) dependent according to the equations below.

$$P(2) + P(1) = 75.2 \pm 4.4$$

$$P(6) + P(5) = 87.7 \pm 2.7$$

$$P(10) + P(9) = 87.7 \pm 3.9$$

$$P(14) + P(13) = 85.6 \pm 4.1$$

The SAAM program allows dependence relations to be entered subject to certain statistical constraints, as opposed to the more commonly used absolute constraints. The calculated values for the P(1) shown in Table III do not satisfy the above four equations exactly, but are consistent with these equations within the statistical ranges allowed. The slight differences are the result of the requirement to produce the best fit of the tracer data in Fig. 3 yet remain consistent with these dependence relations.

(*3*) Knowledge of the percent of total VLDL apo B in the NZW rabbit constituted by large VLDL apo B allows P(1) to be dependent according to the relation

$$P(1) = 5.26 \times P(5)/100.$$

As shown in Table III, P(5) is calculated to be 83.8% of the apo B in large VLDL in the NZW rabbits.

(*4*) The ratios of fast B,E particles to fast B particles in total VLDL in NZW and WHHL rabbits are equal to those same ratios seen in large VLDL where these ratios are easily resolvable from the tracer data. These ratios allow P(3) and P(11) to be made dependent according to the relations

$$P(3)/P(1) = P(7)/P(5)$$

$$P(11)/P(9) = P(15)/P(13).$$

These relations assume that either all or a representative fraction of fast B,E and B particles in total VLDL are found in large VLDL in both NZW and WHHL rabbits.

(*5*) The ratio of fast B,E particles in total VLDL in NZW rabbits to fast B,E particles in total VLDL in WHHL rabbits is given by the same ratio in large VLDL. This assumption allows P(9) to be made dependent according to the relation

$$P(9)/P(1) = P(13)/P(5).$$

The above equation assumes that the fraction of total VLDL apo B isolated in the preparation of large VLDL in the WHHL rabbits is the same as the comparable fraction in the NZW rabbits. This assumption is based on the similarity of the distributions of particle diameters from large VLDL in NZW and WHHL rabbits obtained from electron photomicrographs.

(*6*) The ratio of fast to slow B particles in large VLDL from WHHL rabbits is assumed equal to the ratio of fast to slow B,E particles in large VLDL from WHHL rabbits. This assumption allows P(15) to be made dependent according to the relation

$$P(15)/P(16) = P(13)/P(14).$$

This equation, which allows the unique estimation of the fractional turnover rate of fast B particles (fractional irreversible loss from plasma plus fractional rate of conversion to fast B,E particles) in WHHL rabbits, is the most difficult to substantiate metabolically. However, even if this constraint were to be in error by a wide margin, total apo B transport in the WHHL rabbit would hardly be affected, given the small contribution of this pathway to total apo B transport as seen in Fig. 5.

(*7*) The remaining initial conditions, P(5), P(7), and P(13) are uniquely resolvable with the other adjustable parameters subject to the above mentioned constraints and tracer data shown in Fig. 3.

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