# Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [$^3$H]leucine tracer

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**Abstract** The kinetics of apolipoprotein B (apoB) were measured in seven studies in heterozygous, familial hypercholesterolemic subjects (FH) and in five studies in normal subjects, using in vivo tracer kinetic methodology with a [$^3$H]leucine tracer. Very low density (VLDL) and low density lipoproteins (LDL) were isolated ultracentrifugally and LDL was fractionated into high and low molecular weight subspecies. ApoB was isolated, its specific radioactivity was measured, and the kinetic data were analyzed by compartmental modeling using the SAAM computer program. The pathways of apoB metabolism differ in FH and normal subjects in two major respects. Normals secrete >90% of apoB as VLDL, while one-third of apoB is secreted as intermediate density lipoprotein IDL/LDL in FH. Normals lose 40–50% of apoB from plasma as VLDL/IDL, while FH subjects lose none, metabolizing all of apoB to LDL. In FH, there is also the known prolongation of LDL residence time. The leucine tracer, biosynthetically incorporated into plasma apoB, permits distinguishing the separate pathways by which the metabolism of apoB is channeled. ApoB synthesis and secretion require 1.3 h. ApoB is secreted by three routes: *1*) as large VLDL where it is metabolized by a delipidation chain; *2*) as a rapidly metabolized VLDL fraction converted to LDL; and *3*) as IDL or LDL. ApoB is metabolized along two pathways. The delipidation chain processes large VLDL to small VLDL, IDL, and LDL. The IDL pathway channels nascent, rapidly metabolized VLDL and IDL particles into LDL. It thus provides a fast pathway for the entrance of apoB tracer into LDL, while the delipidation pathway is a slower route for channeling apoB through VLDL into LDL. LDL apoB is derived in almost equal amounts from both pathways, which feed predominantly into large LDL. Small LDL is a product of large LDL, and the major loss of LDL-apoB is from small LDL. Two features of apoB metabolism in FH, the major secretory pathway through IDL and the absence of a catabolic loss of apoB from VLDL/IDL, greatly facilitate measuring the metabolic channeling of apoB into LDL.—**Fisher, W. R., L. A. Zech, L. L. Kilgore, and P. W. Stacpoole.** Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [$^3$H]leucine tracer. *J. Lipid Res.* 1991. **32:** 1823–1836.

**Supplementary key words** compartmental modeling • tracer kinetics

In transporting lipids, plasma lipoproteins are subjected to a series of enzymatic reactions and physical-chemical processes that have been examined in detail in vitro. The physiology of the plasma lipid transport system may also be examined in vivo utilizing tracer kinetic methodology. Measurements obtained in kinetic studies translate the knowledge of lipoprotein metabolism at the molecular level into understanding the altered physiology occurring in specific diseases.

Familial hypercholesterolemia (FH) is a common disorder in which lipoprotein cholesterol delivery to cells is impaired due to dysfunction of the apolipoprotein B,E receptor (1). The metabolic consequences of this abnormality have been examined in vivo with the use of radioiodinated apoB tracers, and repeated studies have demonstrated reduced clearance of LDL-apoB (2). Studies have also quantitated the importance of the apoB,E receptor pathway and identified alternate non-receptor-mediated routes for LDL catabolism (1, 2). A protein labeled exogenously with radioiodine traces catabolic events but cannot be used to directly examine apolipoprotein biosynthesis and secretion. In contrast, an endogenous tracer, such as [$^3$H]leucine, permits evaluation of these latter processes. We now report the results of such an investigation of the pathways of apolipoprotein B (apoB) secretion and its metabolic channeling in subjects heterozygous for FH and in normal controls.

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Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; FH, familial hypercholesterolemia; L-LDL, large LDL; S-LDL, small LDL.

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## SUBJECTS

This investigation was approved by the Institutional Review Board of the University of Florida Health Center. Kinetic studies were performed on seven subjects with heterozygous FH, diagnosed by established clinical criteria (1), and five studies were done on normal subjects. **Table 1** details the clinical features of the subjects whose data are presented in this report. Investigations were performed while the subjects were maintained in metabolic balance on the Clinical Research Center of Shands Hospital. Initial studies on all subjects were performed after discontinuation of lipid-lowering drugs for at least 1 month. One week prior to tracer injection each subject began a constant diet adjusted to maintain stable body weight: 20 g fat, a leucine intake of 5–6 g, protein provided to achieve the desired leucine intake, and carbohydrate adjusted to achieve a stable weight. Subjects were admitted for final diet stabilization 1 week prior to an intravenous bolus injection of 5 $\mu$Ci/kg of [$^3$H]leucine, and 20 blood samples were obtained over 13 days. Beginning 12 h before the tracer injection and continuing for a total of 48 h, subjects received a liquid feeding, by nasogastric infusion, of composition similar to their diet. This low-fat continual feeding, designed to minimize chylomicron formation, was administered to provide a constant level of nutrient intake during the period of frequent blood sampling. Thereafter, blood samples were drawn after a 10-h fast.

## METHODS

### Lipoprotein and apolipoprotein isolation

Specific radioactivity of apolipoprotein B (apoB) from isolated lipoproteins was determined on repetitive blood samples drawn after tracer injection at the times indicated in the figures. At each time, 30 ml of blood was drawn and iced for lipoprotein recovery. Each tube contained 6.0 mg sodium azide, 6.0 mg soybean trypsin inhibitor, 3.0 mg merthiolate, 45 mg EDTA, and 39 mg L-leucine. Five ml of blood was drawn at the same times for isolation of plasma free leucine and measurement of its concentration and specific radioactivity (3).

Plasma for lipoprotein isolation was separated and 0.25 g/ml of sucrose was added prior to freezing to prevent denaturation (4). When thawed the samples were filtered, 4 g/ml of KBr was added to adjust the density to 1.24 g/ml, and 15 ml was layered beneath 25 ml of a 1.006 g/ml KBr solution in a 40-ml centrifuge tube. Samples were then centrifuged 3 h at 50,000 rpm in a Beckman vertical rotor at 5°C in a modification of a method by Chung et al. (5). The apoB-containing lipoproteins were recovered dropwise from the upper portion of the tubes, and the relative protein content of each fraction was determined by a fluorescamine assay (6). As reported by Chung et al. (5) and confirmed by us with analytical ultracentrifugation and with SDS-polyacrylamide electrophoresis, the method readily resolved VLDL and LDL. These fractions were isolated, but IDL was not recovered since the quantity of IDL-apoB present in 30 ml of blood was inadequate for measurement of specific activity using the [$^3$H]leucine tracer. Sucrose (0.2 g/ml) was then added to each fraction, and the samples were refrozen. Subsequently, groups of samples were thawed, dialyzed against phosphate-buffered saline, and the lipids were extracted with diethyl ether–ethanol 8:1. The apoB was separated by preparative polyacrylamide electrophoresis using 4% gels with an SDS-imidazole buffer, and apoB was subsequently electroeluted (7). The technique resolves apoB-100 from apoB-48, and only the former was isolated. The

TABLE 1. Clinical data

| Studies | Sex | Age | Wt | Total Chol | HDL-Chol | LDL-Chol | Triglycerides |
|---|---|---|---|---|---|---|---|
| | | *yr* | *kg* | | | *mg/dl* | |
| Normal controls | | | | | | | |
| N I | M | 45 | 73 | 217 | 33 | 152 | 129 |
| N II | M | 46 | 70 | 201 | 37 | 161 | 109 |
| N III | M | 61 | 92 | 164 | 32 | 102 | 137 |
| N IV | M | 33 | 61 | 169 | 45 | 102 | 125 |
| N V | M | 32 | 85 | 169 | 33 | 92 | 217 |
| FH controls | | | | | | | |
| FH I | M | 45 | 85 | 506 | 29 | 432 | 224 |
| FH II | F | 53 | 65 | 438 | 45 | 369 | 120 |
| FH III | F | 39 | 77 | 644 | 60 | 520 | 160 |
| FH IV | M | 52 | 68 | 228 | 41 | 157 | 148 |
| FH V | F | 54 | 55 | 564 | 67 | 481 | 82 |
| FH VI | F | 64 | 57 | 386 | 39 | 305 | 211 |
| FH VII | M | 48 | 66 | 323 | 44 | 261 | 90 |

Lipid determinations were performed by standard methods in the clinical laboratory of the Shands Hospital, and LDL-cholesterol was calculated by the Friedewald equation (8).

recovered apoB was measured using a fluorescamine assay standardized against purified apoB that was quantitated by amino acid analysis. Samples were counted to a 2.5% accuracy using an alkaline protein solubilizer and a toluene-scintillator solution (Solulene 350 and Permablend 3, Packard).

Apolipoprotein B immunoassays on plasma and on lipoprotein fractions were unreliable for a number of the subjects. Accordingly, for most subjects an initial estimate of apoB concentration was obtained by multiplying the LDL-cholesterol concentration, determined by the Friedewald equation, by the ratio of apoB to total cholesterol found in normal LDL (8). These approximate estimates of apoB mass were used in the preliminary analysis of the kinetic data. By contrast, the specific radioactivity measurements of apoB isolated from individual lipoprotein fractions were reproducible and accurate, and for each study 40 to 60 individual apoB samples were measured. For this reason the definitive computer analyses of the tracer data were performed using apoB specific activity data rather than total radioactivity.

### LDL fractionation

As operationally defined, LDL comprises lipoproteins isolated over a wide density and molecular weight range, but LDL from FH subjects is relatively homogeneous in physical size as compared to LDL from hypertriglyceridemic individuals (9). To address the question of metabolic heterogeneity of LDL, in 14 subjects the LDL separated during vertical ultracentrifugation was fractionated into higher and lower molecular weight subspecies, hereafter large and small LDL (L-LDL and S-LDL), by dividing the LDL band at the tube having the peak lipoprotein concentration into two subfractions, comprising the leading and the trailing edges of the LDL peak.[2] Specifically, the apolipoprotein concentration profile of the LDL, recovered by dropwise fractionation of the centrifuge tube, was measured using either the fluorescamine assay or the absorbance of the solution at 280 nm. In all cases the profile was that of a sharp peak with skewing at the base towards particles of a larger molecular weight. The fractions to the left and to the right of the peak were combined separately after eliminating those that comprised the skewed tail, so as to recover about 85-90% of the lipoprotein comprising the peak. The specific radioactivity of apoB in L-LDL and S-LDL was measured.

The rationale for this fractionation is based upon previous studies with LDL ultracentrifuged in a swinging-bucket rotor, where lipoprotein species of progressively larger size could be recovered by sampling across the LDL peak towards the top of the tube (10). To document the validity of this methodology in the vertical rotor, the LDL from one subject were separated into three fractions across the LDL band. The average molecular weights of the lipoproteins in each fraction were measured by analytical ultracentrifugation and demonstrated again an increase in particle size across the LDL peak. In the kinetic studies LDL was divided into only two fractions. To explore differences in size of the LDL from different subjects, in nine studies aliquots of L-LDL and S-LDL were analyzed by analytical ultracentrifugation, and the molecular weights of the lipoproteins recovered from the leading and trailing edges of the LDL peak were measured.

Measurement of LDL molecular weight was performed by analytical ultracentrifugation by a method adapted from Adams and Schumaker (11), in which lipoproteins were centrifuged, at two concentrations, in KBr solutions of density 1.20 g/ml at 25°C.

### Data analysis

Analysis of the kinetic data was performed by compartmental modeling utilizing the SAAM/CONSAM computer program (12). The kinetic data from each subject were analyzed as specific radioactivity, and apoB transports were calculated from the steady state model solutions. Univariant estimates from the compartmental modeling were compared using Student's *t*-test and paired *t*-tests. Logarithmic transformations of the data were performed as needed to remove skewness.

## THE COMPARTMENTAL MODEL

The compartmental model used in this investigation was derived from that previously reported for hypertriglyceridemic subjects (3, 13). The model, **Fig. 1,** has been modified to analyze the kinetic data in 7 studies in normal subjects and 14 studies in FH subjects. Although only the results of kinetic studies on these subjects under control conditions are reported here, the model was developed and tested on data from the entire study population. In this section we describe the model and provide justification for its topology based upon current experimental data.

The plasma leucine specific activity curve is used as a forcing function. The predominant features of plasma leucine kinetics are a rapid decay of leucine specific activity over several orders of magnitude within the first day after tracer injection and then a flattening of the curve with a long, slowly declining tail determined by the recycling of leucine (3, 14). The tracer disappears from plasma and reappears within newly secreted apoB. This interval, the synthesis time, is represented by four compartments (not shown) terminating in compartment 19,

[2]In the kinetic investigation of LDL heterogeneity, data from subjects studied here, under control conditions, were combined with data obtained when subjects were studied on a high carbohydrate diet (23) and on lovastatin (W. R. Fisher, P. W. Stacpoole, and L. A. Zech, unpublished results).

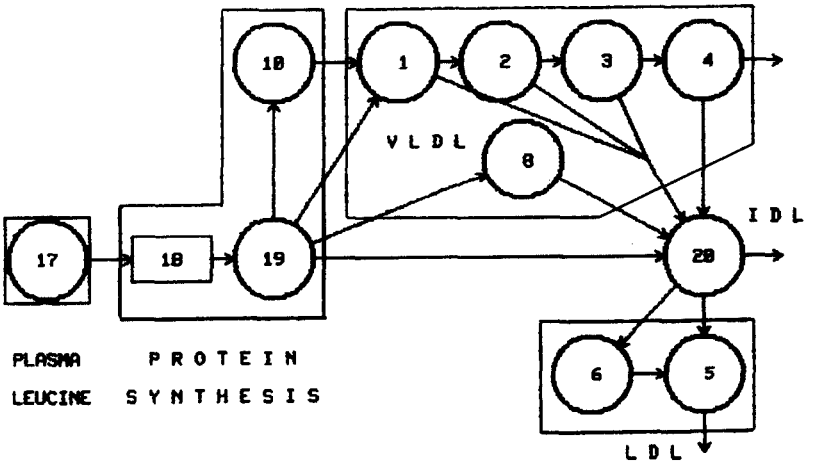



**Fig. 1.** The compartmental model used to fit apoB for the FH heterozygous and normal subjects. Leucine tracer is injected into plasma and, after a delay, it reappears within apoB which may be secreted as large VLDL or as small VLDL or IDL. The secretory pathways to small VLDL and IDL originate in C(19). The secretory pathway to large VLDL and the delipidation chain, C(1)→C(2)→C(3)→C(4), is shown either to enter directly from C(19), as in the VLDL shown in Figs. 2A and B, or through a presecretory delay, C(10), as in Fig. 2C. LDL is modeled as large and small LDL compartments. Both exchange with extravascular pools not shown in this representation of the model.

C(19), and provides a delay equivalent to the minimum time required for synthesis and secretion of apoB.

The appearance of the apoB protein in plasma was measured in VLDL and in large and small LDL. Tracer kinetics of apoB in VLDL took three forms (**Fig. 2**). In some of the FH subjects studied under basal conditions, the tracer entered and departed VLDL rapidly and could be fit to a single compartment with a rapid input and loss, C(8), presumably reflecting a predominant secretion of VLDL particles that are rapidly metabolized to IDL (Fig. 2A). Alternatively, it could be represented by a fast turning-over delipidation chain, [C1→C2→C3→C4→] (13). In others, however, the tracer had a longer residence time in VLDL, requiring a delipidation chain to represent the slower VLDL turnover (Fig. 2B). Another feature of VLDL kinetics is the necessity for an additional apoB secretory delay required in subjects having a further broadening of the VLDL peak (Fig. 2C). The delay in the metabolism of the tracer, giving rise to this broad peak, cannot be accommodated by simply prolonging the residence of the tracer within VLDL; to do so would necessitate a physical mass of apoB far in excess of that present within VLDL. These VLDL are modeled satisfactorily only by providing an early secretory input to VLDL, C(19)→C(8), to fit the sharp rise in the tracer data, plus a delayed input required to sustain the specific activity of the broadened VLDL peak, C(19)→C(10)→C(1), which is attributed to a secretory delay. This third pathway implies that a portion of large VLDL-apoB particles have a prolonged hepatic assemblage time prior to their secretion.

In most subjects, the major portion of apoB entering the delipidation chain is metabolized along this pathway. An alternate route, shunting apoB from the delipidation chain to IDL or LDL, has been demonstrated in studies using radioiodinated VLDL-apoB tracers (15). Such a pathway is resolved with some difficulty using [$^3$H]leucine because of the recycling of the tracer and especially so since IDL-apoB specific activity was not measured in our studies (see below).

The presence of "compartment 21," a slowly turning over VLDL compartment into which a portion of apoB is siphoned from the delipidation chain and then directly lost from plasma, is another feature of VLDL kinetics when studied with radioiodinated VLDL (16). This pathway has been invoked to explain a tail in the VLDL decay, evident at about the end of the first day, with radioiodinated VLDL tracers. The kinetics of leucine recycling makes a large contribution to the shape of this portion of the VLDL kinetic curve, and compartment 21 cannot be specifically evaluated. The pathway through this compartment is reportedly a prominent feature of patients with hypertriglyceridemia, as found in Types III and IV hyperlipoproteinemia, but is not thought to contribute significantly to VLDL-apoB metabolism in normal or FH subjects (16). In our analysis apoB loss by this pathway would be measured as a portion of the total quantity of this apolipoprotein lost from plasma as either VLDL or IDL.

IDL kinetics were not measured experimentally because of inadequate labeling, by the leucine tracer, of the small quantity of this lipoprotein in these subjects. IDL, however, has been previously isolated, physically and chemically characterized, and its metabolic properties have been investigated with both radioiodine and leucine tracers (2, 3, 15, 17–21). In the present studies IDL is included within the model to meet three requirements. *1*) It provides a needed delay between the disappearance of tracer from VLDL and its reappearance within LDL. *2*) In the FH heterozygotes there is a requirement for input of additional LDL-apoB tracer, whose secretion cannot be accommodated within the VLDL, but which appears within the LDL-apoB pool after a sufficiently long delay that it appears unlikely to be nascent LDL. *3*) In our normal subjects a major portion of apoB is lost from the system as VLDL and/or IDL, and in keeping with

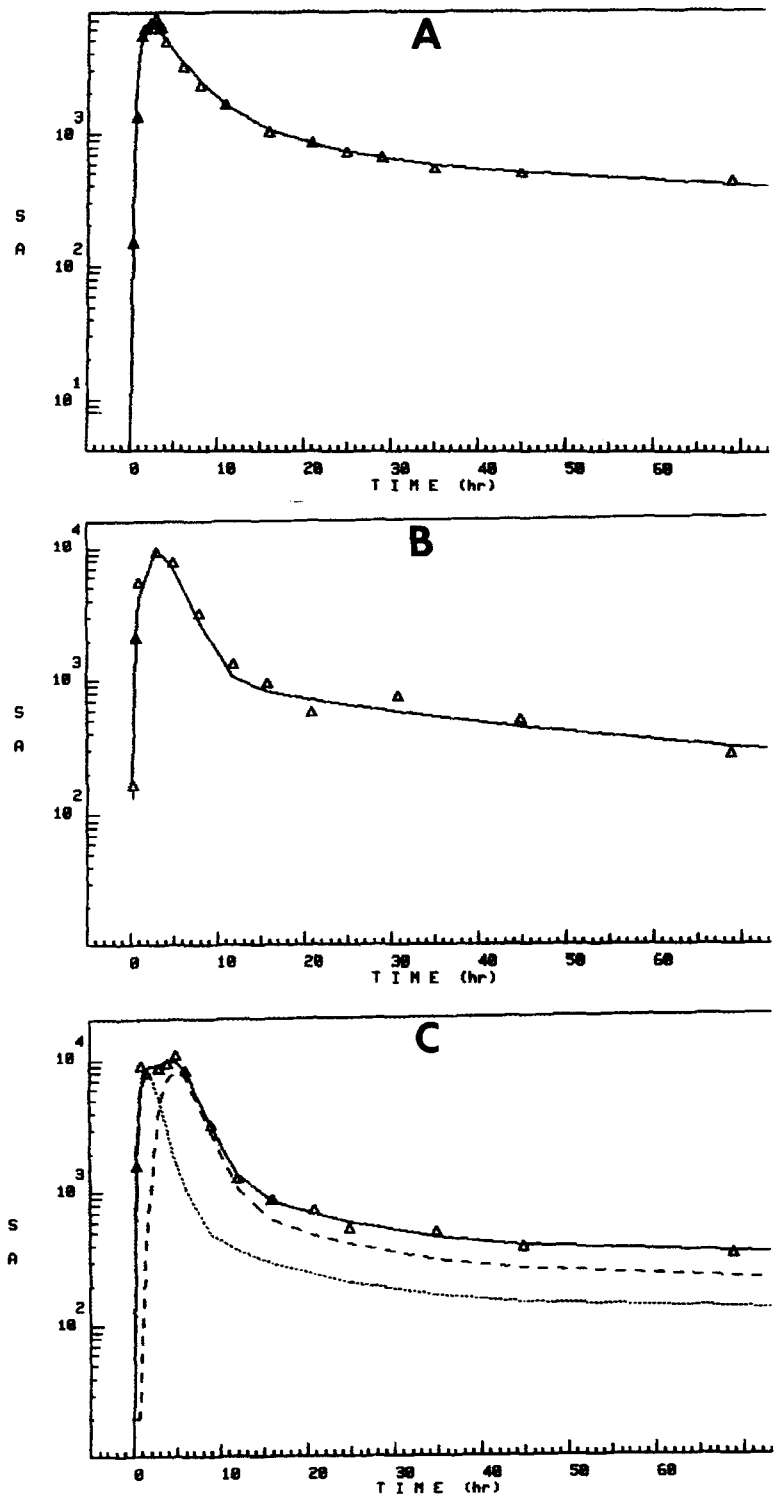



**Fig. 2.** Kinetic data from three subjects showing differences in the metabolism of VLDL-apoB. In each panel the computer-generated fit of the model to the experimental data (△), dpm/mg apoB, is shown by the solid line. A: A rapidly labeled, rapidly decaying VLDL peak is fit by a single compartment, C(8), but could also be fit by a fast turning over delipidation chain, (see also Fig. 4). B: A more slowly turning over VLDL requiring a delipidation chain, C(19)→[C(1)→C(4)] to fit the broad peak produced by the prolonged residence of the tracer. C: A further broadening of the VLDL peak seen in a subject receiving a 91% carbohydrate diet. To fit this peak an early input of tracer is required, C(8), followed by additional tracer entering the VLDL delipidation chain which, however, is delayed in its appearance in plasma, [C(19)→C(10)→[C(1)→C(4)]. By using this presecretory delay, it is possible to fit both the radioactivity and the mass of VLDL-apoB. The kinetics of the delipidation chain are shown by the dashed line and of C(8) by the dotted line.

TABLE 2. Secretion rate (mg/kg/day), fractional transport, residence time (h), and mass (mg) of apoB for individual subjects under control conditions

| Subject | ApoB Synthesis R.T. | ApoB Secretion | ApoB Secretory Delay C(19)→C(10)→C(1) | | VLDL | | | VLDL/IDL Loss FR.TR | IDL/LDL Secretion FR.TR | LDL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | R.T. | FR.TR. | R.T. | FR.TR. | ApoB Mass | | | R.T. | FR.TR | ApoB Mass[a] |
| | *h* | *mg/kg/day* | *h* | | *h* | | *mg* | | | *h* | | *mg* |
| Control: normal | | | | | | | | | | | | |
| N1 | 2.07 | 36.70 | 0.00 | 0.00 | 2.50 | 0.93 | 311.00 | 0.43 | 0.07 | 38.50 | 0.57 | 2140.00 |
| N2 | 1.53 | 118.00[b] | 3.00 | 0.66 | 0.56 | 0.94 | 192.00 | 0.83 | 0.06 | 49.00 | 0.17 | 2670.00 |
| N3 | 1.03 | 22.80 | 0.67 | 0.34 | 1.17 | 0.73 | 170.00 | 0.09 | 0.27 | 29.50 | 0.90 | 2000.00 |
| N4 | 1.23 | 26.30 | 0.00 | 0.00 | 3.44 | 1.00 | 147.00 | 0.57 | 0.00 | 52.10 | 0.48 | 1980.00 |
| N5 | 1.03 | 33.40 | 1.00 | 0.61 | 4.59 | 1.00 | 550.00 | 0.68 | 0.00 | 51.00 | 0.28 | 1960.00 |
| Mean | 1.38 | 29.80 | 0.93 | 0.32 | 2.45 | 0.92 | 274.00 | 0.52 | 0.08 | 44.00 | 0.48 | 2150.00 |
| SD | 0.44 | 6.37 | 1.23 | 0.32 | 1.64 | 0.11 | 167.00 | 0.28 | 0.11 | 9.75 | 0.28 | 299.00 |
| Control: FH | | | | | | | | | | | | |
| FH1 | 1.47 | 17.70 | 0.00 | 0.00 | 9.70 | 0.61 | 357.00 | 0.00 | 0.39 | 147.00 | 1.00 | 9240.00 |
| FH2 | 0.63 | 9.64 | 0.60 | 0.02 | 19.00 | 0.23 | 122.00 | 0.00 | 0.77 | 227.00 | 1.00 | 6130.00 |
| FH3 | 2.01 | 45.70 | 0.00 | 0.00 | 1.11 | 0.28 | 42.00 | 0.00 | 0.73 | 68.30 | 1.00 | 9700.00 |
| FH4 | 1.08 | 17.90 | 0.00 | 0.00 | 4.50 | 0.98 | 239.00 | 0.00 | 0.02 | 55.20 | 1.00 | 2800.00 |
| FH5 | 1.22 | 21.60 | ND | ND | ND | 0.68 | ND | 0.00 | 0.33 | 91.50 | 1.00 | 4570.00 |
| FH6 | 1.03 | 14.30 | 0.00 | 0.00 | 8.31 | 0.89 | 255.00 | 0.00 | 0.11 | 182.00 | 1.00 | 3830.00 |
| FH7 | 1.00 | 29.40 | 0.97 | 0.68 | 0.77 | 0.72 | 57.00 | 0.00 | 0.28 | 53.70 | 1.00 | 4340.00 |
| Mean | 1.21 | 22.30 | 0.26 | 0.12 | 7.22 | 0.63 | 179.00 | 0.00 | 0.37 | 118.00 | 1.00 | 5800.00 |
| SD | 0.44 | 11.90 | 0.42 | 0.28 | 6.81 | 0.28 | 125.00 | 0.00 | 0.29 | 68.50 | 0.00 | 2700.00 |
| Significance of difference (Normal vs. FH) | NS | NS | NS | NS | NS | $P<0.03$ | NS | $P<0.01$ | $P<0.01$ | $P=0.01$ | $P<0.01$ | $P=0.01$ |

Abbreviations: FR.TR., fraction of total apoB secretion transported at each step in the model; R.T., residence time of apoB; ND, not determined.
[a]LDL apoB mass as calculated by the model from the kinetic data.
[b]This value was omitted from the statistical analysis.

findings in hypertriglyceridemic subjects, IDL is an important site for this loss to occur (3, 17–21). Thus, based on observations in which IDL has been isolated and its kinetics have been studied, all of these properties attributed here to IDL have been demonstrated. Because the kinetic data on FH and normal subjects required a compartment with the properties of IDL, C(20) has been introduced in the model to meet this need.

In those studies where LDL was fractionated into high, C(6), and low, C(5), molecular weight subfractions, the kinetic curves indicate that labeling and decay of C(6) precedes that of C(5); hence, they are modeled as precursor and product, respectively. Each fraction, however, receives tracer from C(20). In all but one study it was possible to assign the entire loss of apoB from LDL to C(5).

ApoB is also known to be present within interstitial fluid where it is largely present as LDL, with an average interstitial fluid/plasma concentration ratio for apoB in the range of 0.08 (22). The inclusion of such extravascular pools of exchangeable LDL-apoB within the model for both plasma LDL pools is thus consistent with the physiology of apoB. The compartments are also used to fit the dip in the LDL-apoB curve that commonly occurs between 5 and 20 h; however, the kinetics of these exchanges cannot be determined with any assurance using the leucine tracer.

## RESULTS

### ApoB kinetics in normal and FH subjects[3]

**Table 2** presents data on apoB production and its metabolism in VLDL and LDL for normal and FH subjects studied under control conditions. Total apoB secretion (column 2) is the sum of all secretory pathways from C(19). In order to compare the secretory pathways of apoB among the subjects, transports are presented as fractional values defined as the transport along a pathway, R(i,j), divided by total apoB secretion. Total apoB production, as measured with [$^3$H]leucine, varied widely among individuals, reflecting differences in their lipid metabolism. The small number of normolipidemic studies makes meaningful comparisons of total apoB production between normals and FH subjects difficult.

The time required to produce and secrete an apoB-containing lipoprotein was 1.3 (SD 0.42) h, Table 2, column 1 (mean of total population). As seen in Table 2, column 4, a highly variable portion of newly synthesized VLDL-apoB entered plasma after a secretory delay (C19→C10→C1). This delay was seen only with particles

[3]Model-determined values for fractional transfer coefficients, L(i,j), for individual studies may be obtained from Dr. Fisher upon request.

entering the delipidation chain. It was more evident in the normal subjects but became prominent in the FH subjects after induction of triglyceride synthesis by carbohydrate feeding (23). For lipoproteins traversing this pathway, the total time required for apoB secretion was increased by as much as an hour in some subjects (Table 2, column 3).

An important difference in the kinetics of apoB secretion existed between normal and FH subjects (Table 2, columns 6 and 9). In normals virtually all of apoB was secreted as VLDL. In FH subjects, however, two-thirds of apoB was secreted as VLDL and one-third as smaller particles, presumably of the size of IDL or possibly LDL ($P < 0.03$). Furthermore, the residence time for apoB in VLDL appeared to be longer in FH than normal subjects though this difference was not significant (Table 2, column 5).

Half the total apoB produced by normal subjects was lost as VLDL/IDL, while the remainder was metabolized to LDL (Table 2, column 8 and 11, $P < 0.01$). In contrast, all the secreted apoB in FH subjects was metabolized to LDL; thus all the plasma apoB in these subjects was removed by the pathways of LDL catabolism (Table 2, column 11, $P < 0.01$). Because of the loss of apoB as VLDL/IDL in the normal, LDL-apoB transport in the FH was greater (22 mg/kg/day) than in the normal (15 mg/kg/day) (Table 2, columns 2 and 11, $P < 0.01$). As anticipated, the residence time of LDL-apoB was significantly prolonged in FH (Table 2, column 10, $P = 0.01$).

### Metabolic channeling of apoB

In 14 studies the LDL band separated during vertical ultracentrifugation of the plasma was fractionated into larger and smaller molecular weight subspecies (L-LDL and S-LDL). To explore differences in size of the LDL from different subjects, in 9 studies aliquots of L-LDL and S-LDL were analyzed by analytical ultracentrifugation. The molecular weights of the lipoproteins recovered from the leading and trailing edges of the LDL peak from these subjects were measured, and LDL was consistently fractionated into populations of larger and smaller lipoproteins differing in size by a mean of $0.52 \times 10^6$ Da (SD $0.28 \times 10^6$ Da). Size heterogeneity of the predominant species of LDL isolated from normal individuals and FH patients spanned the LDL molecular weight range from 2.5 to $3.6 \times 10^6$ Da as reported previously (24).

**Figs. 3A–C** provides strong evidence that in FH the size heterogeneity of LDL, which resulted from structural differences in the particles (9), was also associated with kinetic heterogeneity. The apoB lipoproteins comprising LDL originated in at least two pathways (Fig. 1). One path, with secretion into C(8) and C(20), provided rapid input of apoB to LDL and determined the steepness of the early rise in its specific activity. The second path, providing a delayed input by way of the delipidation chain, shaped the contour of the LDL peak after its initial ascent. The LDL kinetic curves were fit by channeling all apoB through a single IDL compartment, C(20). However, by using a two-compartmental IDL model, the distribution of apoB entering plasma as large VLDL or as smaller IDL-like particles, can be more readily appreciated (Fig. 3D). Thus the delipidation pathway, which provided a delayed input of apoB to LDL, comprises C1→C4→C22→LDL while the IDL pathway, providing an early apoB input to LDL, comprises C8→C21→LDL. The delipidation pathway transported about one-half of apoB mass as VLDL (**Table 3A**, columns 4 and 9). The average delay along this pathway was 10.2 h (Table 3B, columns 1 plus 2).

The IDL pathway, comprising C(8) and C(21), transports rapidly metabolized VLDL and IDL-apoB destined for conversion to LDL. This pathway provided a total delay of only 4.9 h for apoB entering LDL (Table 3B, columns 3 plus 4). In FH the delay in the IDL pathway was even shorter, since heterozygotes secreted about one-third of total apoB directly as IDL. Fifty-six percent of apoB transport through C(21) consisted of newly secreted apoB (R(20,19)/(R(20,19)+R(8,19)).[4] These lipoproteins, when mixed with the apoB from C(8), further shortened the average residence time of apoB particles traversing the IDL pathway to 3.5 h in the FH subjects,[5] compared to 10.2 h for particles traversing the VLDL delipidation pathway.

Separating LDL into larger and smaller molecular weight fractions, L-LDL and S-LDL, facilitated demonstrating the distribution of apoB entering LDL by way of the VLDL delipidation pathway from that by the IDL pathway. Figs. 3B and C show LDL kinetic data from two studies in which these two major input pathways were easily resolved. Input from the IDL pathway was required to add steepness to the ascending shoulder of both LDL curves. The major flux of apoB entering later through the delipidation pathway provided the delayed input of apoB needed to attain the height of the peak. Fig. 3C shows the model derived inputs to S-LDL of apoB along these two pathways.

This analysis is further illustrated in **Fig. 4** with data from a subject in whom the fitting of VLDL could be achieved by channeling the major apoB input through either C(8) or the delipidation chain. However, since the delay in the appearance of tracer in L-LDL and S-LDL

[4] In this calculation, R(20,19) and R(21,19) are considered to be equal since nascent IDL secretion is all assigned to the IDL pathway, see Table 2, column 9. The fractional transport by R(8,19) is 0.29 (SD 0.35). Data are for the FH control subjects.

[5] (R(20,19)/(R(20,19)+R(8,19)))•RT(C21)+(R(8,19)/(R(20,19)+R(8,19)))•(RT(C8)+RT(C21)), where RT is residence time. See Table 3B and footnote 3.

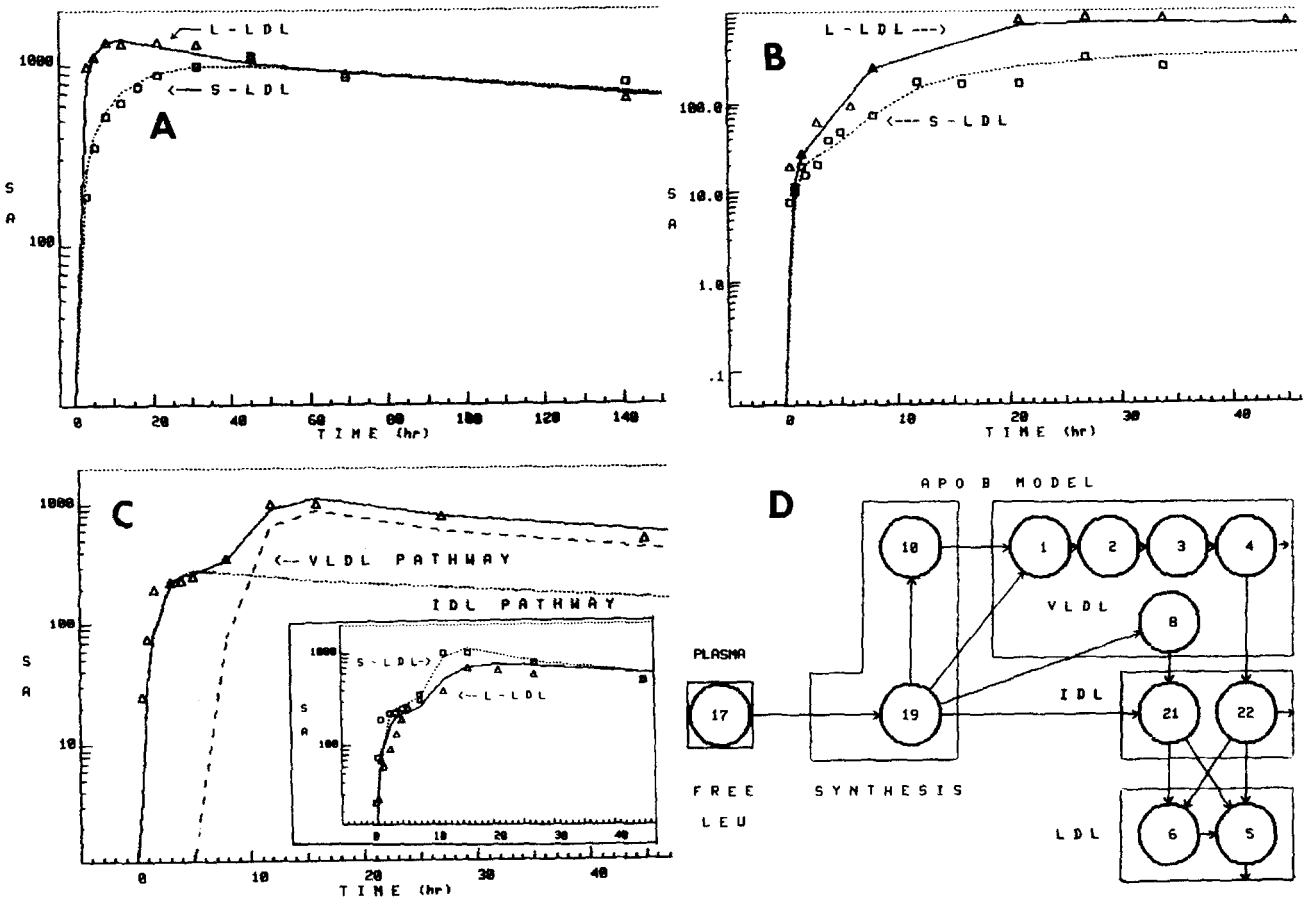



*Fig. 3.* Kinetic curves and computer fits for L-LDL and S-LDL from three studies in FH subjects showing kinetics heterogeneity of LDL-apoB. Data are dpm/mg apoB. A two-compartment IDL model, 3D, permits easier resolution of the data into the IDL pathway, C(19)→C(8)→C(21)→LDL, and the delipidation pathway, C(19)→[C(1)→C(4)]→C(22)→LDL); however, the data can also be fit by a single compartment, IDL model, Fig. 1. A: L-LDL is rapidly labeled by tracer entering plasma primarily via the IDL pathway, while apoB in S-LDL is largely derived from L-LDL in a precursor-product relationship. B: Both L- and S-LDL receive a fast input of apoB by the IDL pathway, while the major apoB flux is delayed en route to LDL via the delipidation pathway, and this input is directed to both LDL pools. C: The S-LDL data from a different study showing the origins of the apoB tracer from the IDL and delipidation pathways. The inset shows the kinetics of both L-LDL and S-LDL. D: An alternate apoB model in which IDL is represented by two compartments, C(21) and C(22), to permit better visualization of the IDL and delipidation pathways. The exchange compartments for LDL are omitted.

differed depending upon which pathway apoB traversed, the kinetics of L-LDL and S-LDL defined the distribution of the tracer between these two pathways. With an endogenous tracer it is thus possible to distinguish and measure the secretion and flux of apoB by these two separate metabolic pathways.

On average, the total flux of apoB to LDL by way of the delipidation and the IDL pathways was approximately equal (Table 3A, columns 4 and 9). ApoB entering through both pathways was primarily directed into L-LDL, as shown by the sum of R(6,21)+R(6,22) which equaled a fractional apoB transport of 0.72 (Table 3A, column 10). The remaining 28% constituted apoB entering S-LDL directly from IDL (Table 3A, column 11), plus that from de novo LDL secretion. Thus, in all subjects L-LDL was the primary precursor of S-LDL, and the fractional transport of apoB from L-LDL to S-LDL, R(5,6), was 0.69 (Table 3, column 12). The difference of 3% (0.72–0.69) resulted from catabolism of L-LDL, while 97% of LDL-apoB was catabolized as S-LDL.

## DISCUSSION

### Elements of the apoB model

A major objective in analyzing physiologic data by compartmental modeling is to develop an analog of the physiologic system to facilitate understanding its behavior. Models depicting apoB metabolism have evolved substantially (15, 16, 20, 25, 26) since the initial apoB compartmental model generated by studies in a hypertriglyceridemic subject (13). The model used in this inves-

TABLE 3. Metabolic channeling of apoB[a]

| | A. Fractional transport via specific pathways (average of data from 14 studies) | | | | | |
|---|---|---|---|---|---|---|
| | 1<br>Delipidation Chain | 2<br>VLDL Path→S-LDL R(5,22) | 3<br>VLDL Path→L-LDL R(6,22) | 4<br>Total VLDL→LDL R(LDL,22) | 5<br>Input C(8) R(8,19) | 6<br>Input C(21) R(21,19) |
| Mean all subjects | 0.45 | 0.15 | 0.29 | 0.44 | 0.27 | 0.26 |
| SD | 0.33 | 0.23 | 0.31 | 0.30 | 0.30 | 0.28 |
| | 7<br>IDL→S-LDL R(5,21) | 8<br>IDL→L-LDL R(6,21) | 9<br>Total IDL→LDL R(LDL,21) | 10<br>Total ApoB→L-LDL R(6,21) + R(6,22) | 11<br>Total ApoB→S-LDL R(5,21) + R(5,22) | 12<br>L-LDL→S-LDL R(5,6) |
| Mean all subjects | 0.07 | 0.42 | 0.48 | 0.72 | 0.18 | 0.69[b] |
| SD | 0.12 | 0.31 | 0.32 | 0.28 | 0.21 | 0.27 |
| Significance of difference | (---------$P < 0.01$---------) | | | (----------$P < 0.01$----------) | | |

| | B. Residence time in elements of the apoB model (time in hours) | | | | | |
|---|---|---|---|---|---|---|
| | VLDL Chain | IDL C(22) | VLDL C(8) | IDL C(21) | LDL C(6) | LDL C(5) |
| Mean all subjects | 5.73 | 4.45 | 2.55 | 2.38 | 51.13 | 45.46 |
| SD | 6.32 | 9.22 | 3.8 | 4.28 | 38.67 | 51.66 |

[a]See Fig. 3D for compartment numbering.
[b]The loss of apoB from C(6) equals 0.03.

tigation was developed and tested by analyzing kinetic data from 21 experiments reported here and elsewhere (23). Here we shall compare the physiology of apoB in FH, as revealed by [$^3$H]leucine, to observations reported by others utilizing a radioiodinated apoB tracer.

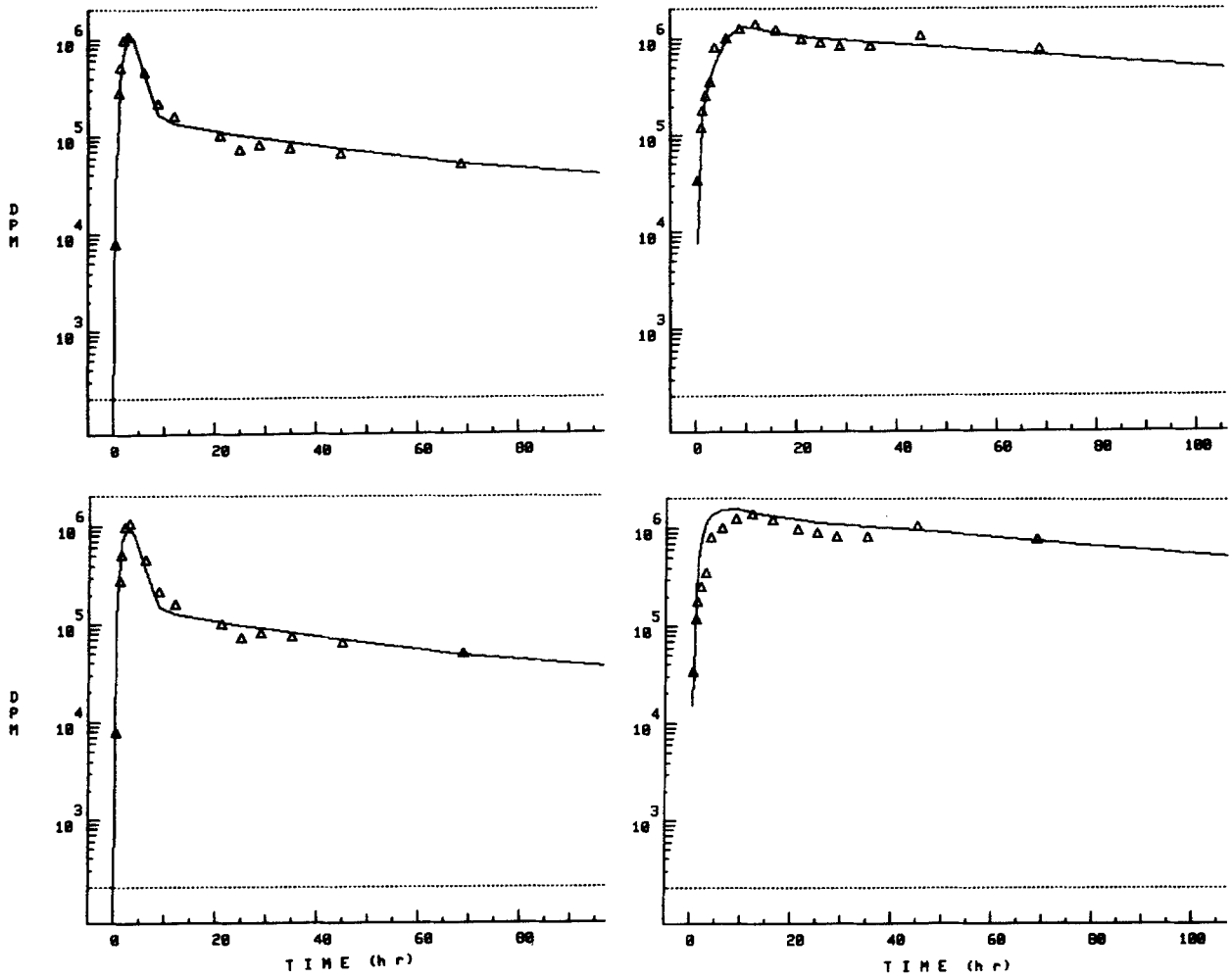
ApoB synthesis and secretion required 1.3 h as measured in vivo with a leucine tracer. In vitro studies using HepG2 cells and chick hepatocytes indicate that synthesis of apoB in the endoplasmic reticulum and assemblage in the Golgi require between 1 and 1.5 h (27, 28). Newly synthesized apoB-containing lipoproteins had a longer intracellular lifetime than many other proteins, including apoA-I (29), and apoB lipoproteins appear to be assembled stepwise in HepG2 cells (30).

The apparent increased secretion of apoB in normals compared to FH subjects was not significant (Table 2, column 2). Total apoB secretion in the seven FH heterozygotes was 22 mg/kg per day, which was only slightly greater than reported using a radioiodinated VLDL-apoB tracer (31).

Three sites of entry of nascent apoB into plasma can be resolved with [$^3$H]leucine. Normal subjects and FH heterozygotes both secreted a major portion of apoB as large VLDL, which traversed the delipidation chain and IDL, before appearing as LDL. In addition, apoB entered a VLDL fraction, C(8), which rapidly labeled and decayed. This fraction is thought to constitute small VLDL or IDL particles isolated within the VLDL class that are rapidly metabolized to IDL. ApoB may follow a third secretory pathway entering plasma distal to VLDL, and in our studies it was initially observed within LDL. Lacking kinetic data on IDL, we cannot determine whether this apoB actually entered plasma as IDL or as LDL.

In normal subjects most of apoB is secreted as VLDL, either by the delipidation chain or through C(8), as is the case in hypertriglyceridemic individuals (3). By contrast, in FH 40% of apoB is secreted as smaller particles, either IDL or LDL, while 60% is secreted as VLDL (Table 2, columns 6 and 9). Similar observations have previously been reported using radioiodinated VLDL tracers (32–34).

Beltz et al. (15) have proposed that the nascent IDL/LDL-like lipoproteins produced in FH actually arise from an apoB fraction secreted as large VLDL but which is rapidly shunted through a fast delipidation pathway to LDL. For most FH subjects the large mass of apoB required to fit LDL could rarely be introduced through the VLDL delipidation chain unless turnover was exceedingly rapid. An alternate interpretation, consistent with the known physiology of VLDL lipolysis (35), would be that such nascent VLDL are small, IDL-like lipoproteins. Such a hypothesis is consistent with the known physical properties of IDL, as isolated in the density fraction 1.019–1.006 g/ml (19). These lipoproteins have a hydrated density of 1.004 g/ml and thus also distribute within the class of particles recovered within the 1.006 g/ml supernatant, namely the VLDL fraction. Accordingly, it has




been possible to improve the fit of VLDL in some FH subjects by redirecting a portion of apoB input from C(20) into a rapidly turning over VLDL compartment with a residence time of about 0.1 h (compared to 2-4 h for C(8)), which feeds into C(20). The kinetic data on whole VLDL cannot distinguish between the Beltz hypothesis and that proposed here; however, we prefer to consider the lipoproteins represented by C(8) and C(20) as a continuum of IDL-like particles.

In normal subjects approximately one-half of apoB production is lost as VLDL/IDL (Table 2, column 8), and HTG subjects lose over two-thirds of apoB by this route (3). Such loss has also been demonstrated in normals, hypertriglyceridemics, and FH homozygotes using radioiodinated apoB tracers (21, 36, 37). Yet in none of the FH heterozygotes reported here was a loss of VLDL/IDL apparent. In these individuals, apoB,E receptor dysfunction appeared to prolong VLDL/IDL catabolism, since the residence time of apoB in FH VLDL was 6.4 h, compared to 2.5 h in normals (Table 2, column 5). Shepherd and Packard (2) and Soutar, Myant, and Thompson (17) have focused attention on apoB,E receptor dysfunction causing impaired VLDL/IDL metabolism in FH. However, the absence of the VLDL/IDL catabolic pathway in FH, in contrast to the normal and hypertriglyceridemic states, is a finding not previously recognized in humans but is comparable to similar observations made in the Watanabe Heritable Hyperlipidemic rabbit (38). The normal rabbit catabolizes much of IDL-apoB, but in the receptor-deficient rabbit decreased IDL catabolism occurs, with increased conversion of IDL to LDL, attributed to the receptor defect. It is, however, the loss of VLDL and IDL in normal individual and the appearance of newly secreted IDL/LDL-apoB in FH that account for the greater quantity of LDL-apoB transport in FH ($P < 0.01$) (Table 2, column 11; and references 32, 34, 37, 39, 40). The actual LDL-apoB transport rate in FH of 22 mg/kg per day measured with [$^3$H]leucine is in close agreement with values reported using radioiodinated LDL (31).

The prolonged residence time of LDL-apoB in FH heterozygotes was initially reported by Langer, Strober, and Levy (41). Our data with the leucine tracer are con-

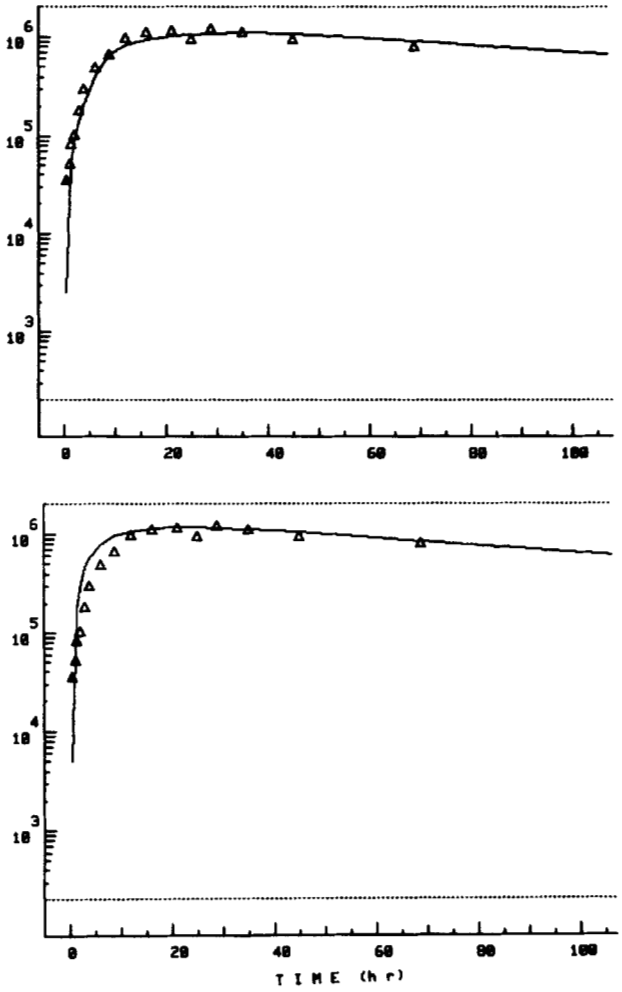



**Fig. 4.** A demonstration of the importance of LDL kinetics in assessing the secretory pathways for VLDL-apoB. Two approximately equivalent fits of the same VLDL, left upper and lower panels, but differing in that in the upper three panels the major flux of apoB enters through the delipidation pathway while in the lower three panels a larger apoB flux enters through small VLDL, C(8), and the IDL pathway. The kinetic properties of L-LDL, middle panels, and S-LDL, right panels, provide the information needed to determine the proper flux of apoB entering VLDL via the IDL and the delipidation pathways. Thus, in the upper panels L-LDL and S-LDL are satisfactorily fit while in the lower panels the larger tracer input to C(8) does not fit LDL. Data are total dpm of apoB in plasma. Parameters for the two computer solutions are as follows:

| | Upper Panels | Lower Panels |
|---|---|---|
| L(10,19) $h^{-1}$ | 1.75 | 1.25 |
| L(8,19) $h^{-1}$ | 0.50 | 2.00 |
| R(10,19) mg/h | 62 | 45 |
| R(8,19) mg/h | 18 | 72 |

sistent with those of others who have confirmed the initial findings using radioiodinated LDL tracers (36, 39).

### LDL heterogeneity and metabolic channeling of apoB

Most studies of LDL heterogeneity have been performed in patients with hypertriglyceridemia or combined hyperlipoproteinemia, where physical heterogeneity of LDL is more overt, or in normal subjects (3, 20, 42, 43). We previously reported studies in hypertriglyceridemic subjects using a leucine tracer, and a precursor–product relationship was readily demonstrable between large and small LDL (3). Those subjects, however, clearly had at least two different sized subspecies of lipoproteins within the LDL class. In contrast, the predominant mass of LDL lipoproteins in FH heterozygotes comprise a family of subspecies of very similar size (9). Yet these lipoproteins may be fractionated by size, and our data show that the larger and smaller subspecies, L-LDL and S-LDL, are also metabolized differently.

Prior investigations of metabolic heterogeneity in FH heterozygotes and normals used LDL fractionated by ultracentrifugation, and radiolabeled large and small LDL subfractions were reinjected as tracers (42–45). In those reports a predominant flux of large to small LDL was evident, but recovery of some small LDL tracer in the large LDL fraction was also observed. Although the metabolism of apoB has long been considered to be unidirectional, those observations suggest the existence of recycling within the major metabolic flow of apoB from lipid-enriched to lipid-depleted particles, probably catalyzed by lipid transfer proteins. The kinetics of apoB examined with the [$^3$H]leucine tracer, however, do not demonstrate recycling of LDL-apoB in FH subjects, or in our previous studies with hypertriglyceridemic subjects (3). In vitro data suggest that lipid core exchange of triglyceride with cholesteryl ester may play an important role in the delipidation cascade by which IDL and larger LDL are thought to be converted to smaller subspecies (46). Such a mechanism, however, need not involve re-

cycling of LDL-apoB itself into larger or lower density particles, since triglyceride and cholesteryl ester have very similar densities and exchange should not appreciably alter the dimensions of the particle (47).

Lipoproteins are dynamic entities, and the constituents are constantly exchanging. Viewed from the perspective of apoB, each molecule exists within a physically definable particle which, in its lifetime, is undergoing a net loss of mass through a process of lipid transfers and triglyceride hydrolysis. ApoB-containing particles may be removed from plasma at various points in their metabolism by binding to cellular receptors. However, apoB-containing lipoproteins that survive to become LDL have lifetimes that are a function of the quantity of lipid with which the particle was initially secreted. Metabolic channeling of apoB can be observed in the kinetics of LDL, since separate large VLDL- and smaller IDL/LDL-derived populations can be distinguished by the times at which their tracers appear in LDL. Most particles entering by both pathways become L-LDL and are metabolized to S-LDL, but some make their initial appearance in S-LDL (see also Teng et al. (42) for studies using radioiodinated tracers). IDL was modeled as two compartments to facilitate tracking the leucine tracer that entered plasma as small particles, i.e., via C(8) or by de novo IDL secretion C(21), from remnants of the VLDL delipidation chain, C(22) (Fig. 3D). The data were then examined to test whether apoB-containing lipoproteins maintained their separate identity as the IDL and delipidation pathways converge en route to LDL. Because C(21) and C(22) can be replaced by a single IDL compartment, C(20), without appreciably compromising the fit of LDL, and since no experimental data on IDL kinetics were obtained, the two-pool IDL model could not be justified. Still, apoB from each of the two pathways apparently retains its identity when it becomes LDL (Fig. 3). In the 12 FH studies in which metabolic channeling was examined, none demonstrated a loss of apoB en route from VLDL to LDL. Unfortunately, the limited data on the two normal subjects do not permit addressing the question of whether the loss of apoB at the VLDL/IDL stage seen in normal and HTG individuals occurs predominantly from the delipidation or the IDL pathway; however, evidence in HTG subjects indicates that the major loss is of large VLDL (42).

In summary, the pathways by which the apoB-containing lipoproteins are metabolized in the FH heterozygote differ from those in normal subjects in the following ways. In the normal, apoB is primarily secreted as triglyceride-rich VLDL and may be directly catabolized or converted to LDL, while, by contrast, in FH one-third of apoB is secreted as IDL/LDL. The regulation of LDL-apoB concentration by direct removal of VLDL/IDL appears to be an important mechanism consistent with the separate roles of apoB as a transporter of triglycerides and of cholesteryl esters. In the FH heterozygote this fork in the metabolic pathway for apoB is not evident. Rather, apoB metabolism in the FH subject is characterized by the total conversion of VLDL remnants to LDL, an increased secretion of IDL/LDL, and a prolonged plasma residence time of LDL.

As in the hypertriglyceridemic patient, metabolic heterogeneity of LDL in FH can be related to heterogeneity in the physical size of these lipoproteins. The kinetic heterogeneity of LDL makes it possible to demonstrate metabolic channeling of apoB. Thus, one fraction of apoB, secreted as large VLDL and metabolized via the delipidation chain, requires almost three times as long to make its appearance in LDL as does a second fraction of apoB that is rapidly metabolized from VLDL and IDL to LDL. The distribution of apoB from these two pathways, into large and small LDL, is an important determinant of the kinetics of these LDL fractions.

Our findings with the leucine tracer thus confirm many of the observations made with radioiodinated apoB tracers. However, they extend these observations in two specific areas: *1*) by clarifying the secretory pathways of apoB; and *2*) by demonstrating that the metabolic channeling of apoB is a primary determinant of the kinetics of LDL. In fact it is a consequence of two of the primary features of apoB metabolism in FH, the major secretory pathway through IDL and the absence of a catabolic pathway from VLDL, that it has been possible to observe the metabolic channeling of apoB into LDL with such clarity.

If apoB, once synthesized, is secreted in particles of differing lipid content and size, which are metabolized along separate metabolic pathways, then an important question is how the metabolism of the apoB-containing lipoproteins along different pathways in plasma is regulated.

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## REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1989. Familial hypercholesterolemia. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. 6th Edition. McGraw-Hill, New York. 1215–1250.
2. Shepherd, J., and C. J. Packard. 1989. Lipoprotein metabolism in familial hypercholesterolemia. *Arteriosclerosis.* **Supp. 1, 9:** I-39–I-42.
3. Fisher, W. R., L. A. Zech, P. Bardaleye, G. Warmke, and M. Berman. 1980. The metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse LDL. *J. Lipid Res.* **21:** 760–774.
4. Fisher, W. R., and V. N. Schumaker. 1986. Isolation and characterization of apolipoprotein B-100. *Methods Enzymol.* **128:** 247–261.

5. Chung, B. H., T. Wilkinson, J. C. Greer, and J. P. Segrest. 1980. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.* **21:** 284–291.
6. Böhlen, P., S. Stein, W. Dairman, and S. Udenfriend. 1973. Fluorometric assay of proteins in the nanogram range. *Arch. Biochem. Biophys.* **155:** 213–220.
7. Kilgore, L. L., J. L. Rogers, B. W. Patterson, N. H. Miller, and W. R. Fisher. 1985. The determination of specific radioactivity of proteins eluted intact from polyacrylamide gels, utilizing a fluorescamine assay. *Anal. Biochem.* **145:** 113–119.
8. Friedewald, W. T., R. I. Levy, and D. S. Frederickson. 1979. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
9. Fisher, W. R. 1983. Heterogeneity of plasma low density lipoproteins manifestations of the physiologic phenomenon in man. *Metabolism.* **32:** 283–291.
10. Hammond, M. G., M. C. Mengel, G. L. Warmke, and W. R. Fisher. 1977. Macromolecular dispersion of human plasma low-density lipoproteins in hyperlipoproteinemia. *Metabolism.* **26:** 1231–1242.
11. Adams, G. H., and V. N. Schumaker. 1969. Rapid molecular weight estimates for low-density lipoproteins. *Anal. Biochem.* **29:** 117–129.
12. Berman, M., and M. Weiss. 1977. SAAM Manual. U. S. Government Printing Office. Department of Health, Education and Welfare Publication No. 78-180. National Institutes of Health, Washington, DC. 200.
13. Phair, R. D., M. G. Hammond, J. A. Bowden, M. Fried, W. R. Fisher, and M. Berman. 1975. A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. *Fed. Proc.* **34:** 2263–2270.
14. Poole, B. 1971. The kinetics of disappearance of labeled leucine from the free leucine pool of rat liver and its effect on the apparent turnover of catalase and other hepatic proteins. *J. Biol. Chem.* **246:** 6587–6591.
15. Beltz, W. F., Y. A. Kesaniemi, B. V. Howard, and S. M. Grundy. 1985. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, Intermediate density lipoproteins, and low density lipoproteins. *J. Clin. Invest.* **76:** 575–585.
16. Berman, M., M. Hall III, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
17. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1982. The metabolism of very low density and intermediate density lipoproteins in patients with familial hypercholesterolemia. *Atherosclerosis.* **43:** 217–231.
18. Teng, B., A. D. Sniderman, A. K. Soutar, and G. R. Thompson. 1986. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J. Clin. Invest.* **77:** 663–672.
19. Fisher, W. R. 1970. Characterization and occurrence of an $S_f$ 20 serum lipoprotein. *J. Biol. Chem.* **245:** 877–884.
20. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
21. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein B apoprotein in man. *J. Clin. Invest.* **61:** 850–860.
22. Sloop, C. H., L. Dory, and P. S. Roheim. 1987. Interstitial fluid lipoproteins. *J. Lipid Res.* **28:** 225–237.
23. Stacpoole, P. W., K. von Bergmann, L. L. Kilgore, L. A. Zech, and W. R. Fisher. 1991. Nutritional regulation of cholesterol synthesis and apolipoprotein B kinetics: studies in patients with familial hypercholesterolemia and normal subjects treated with a high carbohydrate, low-fat diet. *J. Lipid Res.* **32:** 1837–1848.
24. Fisher, W. R., M. G. Hammond, and G. L. Warmke. 1972. Measurements of the molecular weight variability of plasma low density lipoproteins among normals and subjects with hyper-$\beta$-lipoproteinemia. Demonstration of macromolecular heterogeneity. *Biochemistry.* **11:** 519–525.
25. Fisher, W. R. 1982. Apoprotein B kinetics in man: concepts and questions. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 43–68.
26. Le, N-A., H. Ginsberg, and W. V. Brown. 1982. Compartmental models for the study of apoprotein B catabolism in man. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 121–138.
27. Borchardt, R. A., and R. A. Davis. 1987. Intrahepatic assembly of very low density lipoproteins. *J. Biol. Chem.* **262:** 16394–16402.
28. Bamberger, M. J., and M. D. Lane. 1988. Assembly of very low density lipoprotein in the hepatocyte: differential transport of apoproteins through the secretory pathway. *J. Biol. Chem.* **263:** 11868–11878.
29. Ruderman, N. B., A. L. Jones, R. M. Krauss, and E. Shafrir. 1971. A biochemical and morphologic study of very low density lipoproteins in carbohydrate-induced hypertriglyceridemia. *J. Clin. Invest.* **50:** 1355–1368.
30. Boström, K., J. Borén, M. Wettesten, A. Sjöberg, G. Bondjers, O. Wiklund, P. Carlsson, and S-O. Olofsson. 1988. Studies on the assembly of apoB-100-containing lipoproteins in HepG2 cells. *J. Biol. Chem.* **263:** 4434–4442.
31. Kesaniemi, Y. A., G. L. Vega, and S. M. Grundy. 1982. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 182–207.
32. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very-low and low-density lipoproteins in familial hypercholesterolemia. *Atherosclerosis.* **28:** 247–256.
33. Eaton, R. P., R. C. Allen, and D. S. Schade. 1982. Metabolic heterogeneity of low density lipoprotein apoB production in familial hypercholesterolemia: an analytical model solution of tracer data. *J. Lipid Res.* **23:** 738–746.
34. Janus, E. D., A. Nicoll, R. Wootton, P. R. Turner, P. J. Magill, and B. Lewis. 1980. Quantitative studies of very low density lipoprotein: conversion to low density lipoprotein in normal controls and primary hyperlipidemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolemia. *Eur. J. Clin. Invest.* **10:** 149–159.
35. Higgins, J. M., and C. J. Fielding. 1975. Lipoprotein lipase. Mechanism of formation of triglyceride-rich remnant particles from very low density lipoproteins and chylomicrons. *Biochemistry.* **14:** 2288–2292.
36. Packard, C. J., J. L. H. C. Third, J. Shepherd, A. R.

Lorimer, H. G. Morgan, and T. D. Veitch Lawrie. 1976. Low density lipoprotein metabolism in a family of familial hypercholesterolemic patients. *Metabolism.* **25:** 995–1006.

37. James, R. W., B. Martin, D. Pometta, J. C. Fruchart, P. Duriez, P. Puchois, J. P. Farriaux, A. Tacquet, T. Demant, R. J. Clegg, A. Munro, M. F. Oliver, C. J. Packard, and J. Shepherd. 1989. Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. *J. Lipid Res.* **30:** 159–169
38. Kita, T., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1982. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL Rabbits. *Proc. Natl. Acad. Sci. USA.* **79:** 5693–5697.
39. Simons, L. A., D. Reichl, N. B. Myant, and M. Mancini. 1975. The metabolism of the apoprotein of plasma low density lipoprotein in familial hyperbetalipoproteinemia in the homozygous form. *Atherosclerosis.* **21:** 283–298.
40. Bilheimer, D. W., N. J. Stone, and S. M. Grundy. 1979. Metabolic studies in familial hypercholesterolemia. Evidence for a gene-dose effect in vivo. *J. Clin. Invest.* **64:** 524–533.
41. Langer, T. W., W. Strober, and R. I. Levy. 1972. The metabolism of low density lipoprotein in Familial Type II Hyperlipoproteinemia. *J. Clin. Invest.* **51:** 1528–1536.
42. Teng, B., A. D. Sniderman, A. K. Soutar, and G. R. Thompson. 1986. Metabolic basis of hyperapobetalipoproteinemia: turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J. Clin. Invest.* **77:** 663–672.
43. Vega, G. L., and S. M. Grundy. 1986. Kinetic heterogeneity of low density lipoproteins in primary hypertriglyceridemia. *Arteriosclerosis.* **6:** 395–406.
44. Kissebah, A. H. 1987. Low density lipoprotein metabolism in non-insulin-dependent diabetes mellitus. *Diabetes.* **3:** 619–651.
45. Beltz, W. F., S. G. Young, and J. L. Witztum. 1987. Heterogeneity in low density lipoprotein metabolism. *In* Proceedings of the Workshop on Lipoprotein Heterogeneity. U.S. Dept. of Health and Human Services, NIH Pub. No. 87-2646. Kenneth Lippel, editor. 215–236.
46. Deckelbaum, R. J., S. Eisenberg, Y. Oschry, E. Butbul, I. Sharon, and T. Olivecrona. 1982. Reversible modification of human plasma low density lipoproteins toward triglyceride-rich precursors. *J. Biol. Chem.* **257:** 6509–6517.
47. Sata, T., R. J. Havel, and A. J. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.