Metabolic heterogeneity of low density lipoprotein-apoB production in familial hypercholesterolemia: an analytical model solution of tracer data

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Abstract Six subjects with heterozygous familial hypercholesterolemia (FH) (three males, three females) formed the basis for an investigation of the pathways of production of apoprotein B within plasma low-density-lipoprotein (LDL-apoB). Following the intravenous injection of [^{75}Se]selenomethionine as an amino acid tracer, incorporation of the radioactive isotope into the putative precursor of LDL, intermediate density lipoprotein (IDL-apoB), was examined over a 9-day period. The resulting tracer data provided the precursor profile for both the IDL catabolic conversion of LDL as well as for direct synthesis of LDL from amino acids. The fractional conversion rates, (β) for IDL and (α) for amino acids to LDL-apoB, were determined utilizing the two-compartment model for LDL involving both the intravascular and the extravascular pools of apoB. This LDL model was resolved analytically and the parameters α and β were determined so as to give the least squares fit to the LDL tracer data. In this solution, the fractional conversion rates of IDL and of amino acids to LDL-apoB were resolved with a mean fractional residual of $20 \pm 6\%$, which was randomly distributed within the LDL-apoB data throughout the 216 hours of the studies. The steady-state determination of the pathways of LDL-apoB synthesis, based upon this tracer analysis, indicate that IDL accounts for 46%, and amino acids account for 54% of total apoB production within plasma LDL in heterozygous FH. In contrast, in normal subjects, IDL accounts for 86% of total LDL-apoB production. Since the IDL catabolic pathway represents the predominant if not exclusive source of LDL in normal man, this demonstration of a major non-IDL source of LDL in FH broadens the understanding of the pathophysiology of human familial hypercholesterolemia.—**Eaton, R. P., R. C. Allen, and D. S. Schade**. Metabolic heterogeneity of low density lipoprotein-apoB production in familial hypercholesterolemia: an analytical model solution of tracer data. *J. Lipid Res.* 1982. **23:** 738–746.

Supplementary key words [^{75}Se]selenomethionine • intermediate density lipoprotein • very low density lipoprotein

Patients with heterozygous familial hypercholesterolemia (FH) are characterized by elevated circulating plasma beta apoprotein (apoB) within the low density lipoprotein (LDL) (1). Two kinetic abnormalities of LDL-apoB metabolism have been reported: overproduction (1–5), and impaired catabolism (6–8). Indirect evidence suggests that the overproduction of LDL-apoB in FH may involve two potential pathways representing catabolic conversion from circulating very low density lipoprotein (VLDL), and/or direct synthesis of LDL (5, 9). However, specific experimental investigation of this possibility has not been performed. Consistent with the concept of more than one source of LDL-apoB is the reported heterogeneity of the apoprotein isolated from LDL (10–12). Three species with apparent molecular weights of 549,000, 407,000, and 144,500 have been described with unique amino acid compositions (12). Moreover, heterogeneity of intact LDL has been described with differing cholesterol/triglyceride content relating to smaller and larger molecules of lipoprotein (10, 11).

Investigations directed at the source of LDL-apoB have examined the in vivo conversion from VLDL labeled in vitro with ^{125}I-labeled apoB (1, 13–15). By catabolic degradation, this precursor lipoprotein is degraded to LDL via an intermediate (IDL), and represents the major, if not exclusive, source of LDL-apoB in normal man (9, 13–16). Studies in patients with FH have suggested that the conversion of VLDL to LDL is insufficient to account for the total LDL turnover in this disease state (5, 9). It has therefore been proposed that direct synthesis of LDL or of its more immediate precursor, intermediate density lipoprotein (IDL), may participate in the overproduction characteristic of FH. In the present study, we investigated the role of IDL in the quantitative production of LDL, relative to that mediated by direct synthesis from amino acid precursors. By injecting [^{75}Se]selenomethionine as a tracer amino acid precursor for apoB synthesis of both IDL and LDL, we examined the simultaneous kinetic characteristics of ^{75}Se-

Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; ^{75}Se-AA, amino acid labeled with [^{75}Se]-selenomethionine; FCR, fractional catabolic rate.

labeled amino acids (^{75}Se-AA) and ^{75}Se-labeled IDL-apoB as potential precursors for ^{75}Se-labeled LDL-apoB. Our data indicate that plasma LDL in heterozygous FH is derived from both catabolism of IDL and from direct synthesis to an essentially equal degree. This observation extends the two reported kinetic abnormalities in FH, of overproduction and impaired catabolism, to include the characteristic of significant direct LDL-apoB synthesis unrelated to the contribution of precursor catabolism.

METHODS

Six heterozygous FH patients described in Table 1 were studied. The subjects were hospitalized in the Clinical Research Unit and received an isocaloric diet with an intake of 38% from carbohydrate, 45% from fat, and 17% from protein. The subjects were fasted for 16 hr prior to the injection of labeled amino acids and for the initial 4 hr following the administration of the isotope. After control blood samples had been drawn, 250 μCi of [^{75}Se]selenomethionine[1] was injected intravenously and serial 20-ml blood samples were drawn for 10 days (16). Disappearance from the plasma of ^{75}Se as the free amino acid was monitored and incorporation into apoB of IDL and LDL was evaluated by quantitative polyacrylamide gel electrophoresis (PAGE) isolation of the apoprotein from the lipoprotein fractions separated by serial ultracentrifugation (16).

The ^{75}Se radioactivity incorporated into apoB isolated from IDL and LDL by polyacrylamide gel electrophoresis was expressed as cpm/ml of plasma, for each 8-ml plasma sample. The LDL data were analyzed using a two-compartment system representing the intra- and extravascular pools characteristic of LDL-apoB distribution in man (1). The fractional conversion rates of ^{75}Se-AA and ^{75}Se-labeled IDL-apoB to ^{75}Se-labeled LDL-apoB were determined using the analytical solution described below.

Plasma ^{75}Se-labeled amino acid evaluation

Free ^{75}Se-labeled amino acid disappearance from the plasma was monitored by counting the radioactivity present in the supernatant solution of a milliliter of plasma from each sample following precipitation with 10% trichloracetic acid. The initial samples were obtained at 5, 30, 60, 120, and 240 min to permit extrapolation of the plasma specific activity to the moment of injection. Potential contamination of isolated apoprotein by free ^{75}Se-labeled amino acid was monitored by the in vitro addition of [^{3}H]methionine as an amino acid tracer to each plasma specimen and counting the ^{3}H present in isolated apoprotein fractions.

Preparation of lipoproteins

Serum lipoproteins were separated by repetitive preparative centrifugation with a 40.3 rotor, at 12°C, in a Beckman Model A ultracentrifuge (18). Eight ml of plasma from each sample was divided and placed in two 7-ml ultracentrifuge tubes and overlayered with 3 ml of normal saline. The very low density lipoproteins were separated in the $d < 1.006$ g/ml fraction of serum after centrifugation for 18 hr. The IDL fraction was then isolated by increasing the solvent density to 1.019 g/ml. The supernatant fraction containing the IDL was removed after 22 hr. The infranant was then taken to a solvent density of 1.063 g/ml, placed under 2 vol of KBr solution of d 1.063 g/ml, and re-centrifuged for 22 hr to isolate the LDL fraction. The lipoproteins were dialyzed for 72 hr against 100 volumes of 0.01% EDTA solution (pH 7.0) at 4°C, the bath being changed every 12 hr. Total protein content within each lipoprotein fraction was determined by the method of Lowry et al. (19) using bovine albumin as the standard.

Nonspecific degradation of apoB was reduced by the immediate addition of sodium azide (0.02%) to serum and to all buffers and ultracentrifugal media to prevent microbial degradation.

Delipidation was performed by a modification of the method of Brown, Levy, and Fredrickson (20) to prevent potential losses of protein into organic extraction solutions (21, 22). Extractions were performed on lyophylized lipoprotein fractions with anhydrous solvents, as previously described (16).

Quantitative polyacrylamide gel electrophoresis

The apoB moiety of the apoprotein of IDL and LDL was quantitatively isolated by preparative polyacrylamide gel electrophoresis. A Canalco Model 120A unit with precast 12% (w/v) acrylamide sodium dodecyl sulfate (SDS) gels (Bio-Rad Laboratories, Richmond, CA) in 0.205 M Tris buffer (pH 6.4) containing 0.1% sodium dodecyl sulfate (w/v) was utilized. Aliquots of SDS-solubilized apolipoproteins were applied to three tubes, and electrophoresis was carried out at 5 mA/tube until the tracking dye reached a distance of 0.4 cm from the end of the gel. For protein staining the gels were exposed to a solution of 0.003% Coomassie brilliant blue in 10% trichloracetic acid and rinsed in 0.001 M HCL. Following electrophoresis, gels were sliced at 3-mm intervals and the ^{75}Se radioactivity contained within each slice was determined. Recovery of applied radioactivity averaged 96 ± 6% from the preparative gels. The labeled apoB

[1] Obtained for human use from American Biomed Corporation.

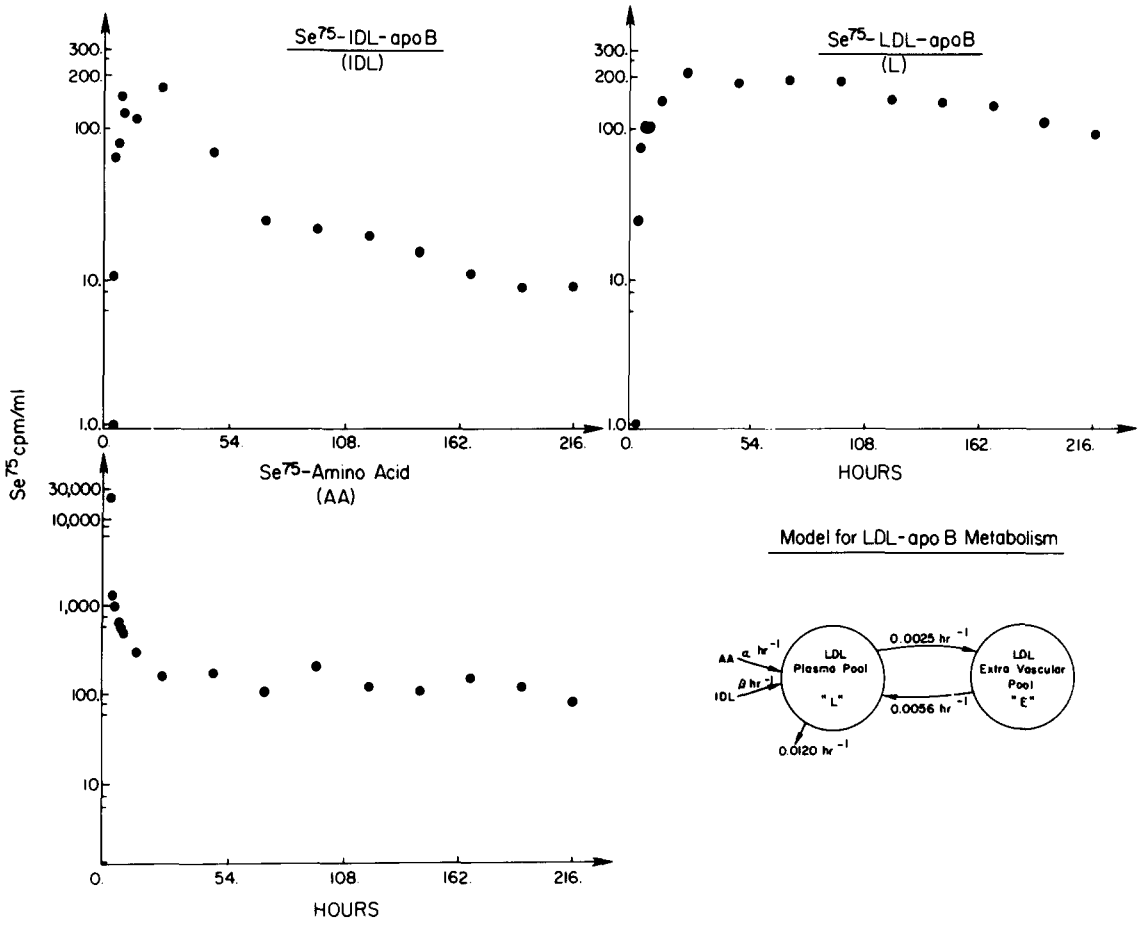

Fig. 1. Plasma ^{75}Se-labeled IDL-apoB, ^{75}Se-amino acid, and ^{75}Se-labeled LDL-apoB radioactivity as a function of time after the intravenous injection of ^{75}Se-amino acid in a subject with heterozygous familial hypercholesterolemia (HT). The two-compartment model (plasma pool and extravascular pool) utilized in the kinetic analysis of the tracer data contains the fractional turnover constants derived from the literature and the fractional conversion constants determined from these studies to define the precursor contributions to LDL-apoB from amino acid (α) and from IDL (β).

was evaluated in the initial 3-mm slice termed Zone 1 by Bilheimer, Eisenberg, and Levy (13), as previously utilized by Berman et al. (15) in their tracer studies.

The ^{75}Se radioactivity within each 8-ml sample incorporated into apoB isolated from IDL and LDL by polyacrylamide gel electrophoresis was expressed as cpm/ml of plasma. The pattern of incorporation of ^{75}Se-labeled amino acid into the plasma free amino acid pool, IDL-apoB, and LDL-apoB is graphically depicted in **Fig. 1** for a typical FH subject (HT). The profiles of incorporation into apoB in the lipoproteins are similar to that reported by Stahelin (23) for ^{75}Se-labeled amino acid incorporation into the total apoprotein of these lipoprotein fractions. A similar sequence of incorporation from ^{3}H-labeled amino acid injection (leucine) into a hyperlipemic subject has also been reported by Phair et al. (24) who monitored apoB isolated by gel filtration chromatography. While quantitative conclusions cannot be made from inspection of the specific activity curves, the timing of isotopic incorporation over the 10 days of observation is compatible with a precursor-product relationship between IDL and LDL. In parallel with the disappearance of ^{75}Se from the plasma amino acid pool, a rapid incorporation is observed within apoB of IDL, attaining a maximum between 4 and 6 hr postinjection of [^{75}Se]selenomethionine as previously reported (16). The incorporation into apoB of LDL demonstrates a more gradual rise to maximum levels by 24–36 hr following the ^{75}Se-labeled amino acid administration (See Fig. 1)

Analytical gel electrophoresis was performed as described above, utilizing 10 μg of apoprotein applied to a gel. Photodensitometric scanning of the gels, following staining with Coomassie brilliant blue, was performed at 602 nm with a scanning spectrophotometer (Gilford Model #240), with digital integration of the peaks obtained as previously described by Kane, Hardman, and Paulus (12). Parallel gel electrophoresis of bovine albumin (Sigma) was used as the standard, with protein recovery averaging 92 ± 4%.

Kinetic evaluation

The relationship, $\frac{d}{dt}(LDL) = \text{Production Rate} - \text{Removal Rate} + \text{Exchange Rate}$, defines the accumulation of amino acid tracer in LDL-apoB. Since both ^{75}Se-labeled amino acid (AA) and ^{75}Se-labeled IDL-apoB are potential precursors for ^{75}Se-labeled LDL-apoB, the production rate can be expressed as Production Rate = $\alpha(AA) + \beta(IDL)$ where the fractional conversion rates, α and β, are to be determined. To define the removal and exchange rates, we have utilized the published data of Bilheimer, Stone, and Grundy (1) which report the average fractional catabolic rate in six heterozygous FH patients as 0.287 day^{-1}. These authors also report that the intravascular portion of LDL-apoB (L) accounts for 69.3% of the total distribution with the remaining apoprotein (E) identified with extravascular sites of exchange. This relationship between the two pools of LDL-apoB can be used to define the flow between the two pools where irreversible loss occurs only from the plasma. The fractional turnover rate from the intravascular to extravascular pool has been reported by Packard et al. (3) in the study of six patients with FH to be 0.0025 hr^{-1}, and so the turnover rate from the extravascular to intravascular pool is 0.0056 hr^{-1}. Thus, removal and exchange of LDL-apoB can be defined by the two-compartment model given in Fig. 1.

With this information, the complete system including production can be visualized by the two-compartment model or by the equivalent mathematical formulation,

$$(A1) \qquad \frac{d}{dt}L(t) = -0.0145L(t) + 0.0056E(t) + \alpha AA(t) + \beta IDL(t),$$

$$(A2) \qquad \frac{d}{dt}E(t) = 0.0025L(t) - 0.0056E(t),$$

$$(A3) \qquad L(t_0) = E(t_0) = 0.$$

The time $t = t_0$ is the time of tracer injection which is taken to be zero.

To find α and β, the fractional conversion rates of AA and IDL respectively, the system of differential equations (*A*) is solved for L(t):

$$(B) \qquad L(t) = \alpha \int_0^t \phi(t-s)AA(s)ds + \beta \int_0^t \phi(t-s)IDL(s)ds$$

where $\phi(u) = 0.8827e^{-0.0159u} + 0.1173e^{-0.0042u}$.

With tracer data for ^{75}Se-AA, ^{75}Se-labeled IDL-apoB, and ^{75}Se-labeled LDL-apoB at the time points $t = t_1, t_2, \ldots, t_n$, equation (*B*) can be formed for each of these t values to obtain the discrete set of equations:

$$(C) \qquad L(t_i) = \alpha \int_0^{t_i} \phi(t_i - s)AA(s)ds + \beta \int_0^{t_i} \phi(t_i - s)IDL(s)ds, \qquad i = 1, 2, \ldots, n.$$

Using the trapezoidal rule to approximate each of these integrals, equations (*C*) result in the following set of n linear equations in the two unknown conversion rates, α and β:

$$(D) \qquad L_i = \alpha\left[\sum_{j=0}^{i-1}(\phi(t_i - t_{j+1})AA_{j+1} + \phi(t_i - t_j)AA_j)\frac{(t_{j+1} - t_j)}{2}\right] + \beta\left[\sum_{j=0}^{i-1}(\phi(t_i - t_{j+1})IDL_{j+1} + \phi(t_i - t_j)IDL_j)\frac{(t_{j+1} - t_j)}{2}\right], \qquad i = 1, 2, \ldots, n$$

where L_k, AA_k, and IDL_k for $k = 1, \ldots, n$ are the observed tracer quantities. At $t = 0$, $IDL_0 = 0$ and AA_0 equals the plasma specific activity determined at the moment of injection. Estimates for α and β were determined as the least squares solution of equations *D*. The Jackknife procedure (17) was applied to correct the estimates for bias and to determine standard errors. The Jackknife estimates in all cases were close to the least squares estimates indicating very little bias was present. The LDL-apoB model solution for the data of the patient (HT) given in Fig. 1, is shown graphically in **Fig. 2.** The appropriateness of the fit is evident from an inspection of the random distribution of the residual (solution minus plasma ^{75}Se-labeled LDL-apoB data) over the 216 hr of study. A quantitative

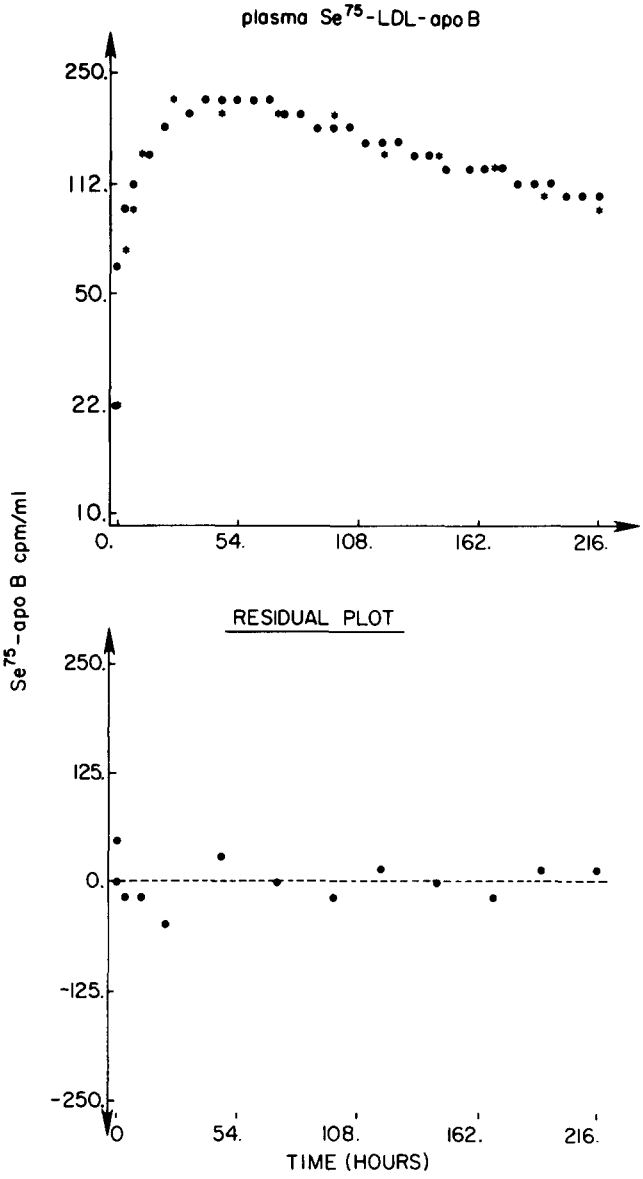

Fig. 2. The upper plot depicts a representative specific activity (cpm/ml versus time) curve for plasma ^{75}Se-labeled LDL-apoB in a heterozygous familial hypercholesterolemic subject (HT). (*) represent the observed data and (●) represent the model predictions. The Residual Plot (below) represents the model prediction minus the observed data, and demonstrates the uniform distribution of the deviation of the solution over the 216 hr of study. The normalized fractional residual for each patient is tabulated in Table 2.

evaluation of this fit may be defined as the normalized fractional residual

$$\left(\sqrt{\sum_{i-1}^{n} (L(t_i) - L_i)^2 / \sum_{i=1}^{n} L_i^2} \right).$$

The steady state turnover of LDL-apoB is calculated as the product of the plasma LDL-apoB concentration (chemically determined) and the FCR (1). The IDL-derived production of LDL-apoB is determined as the product of the plasma IDL-apoB concentration (chemically determined) and the fractional conversion (β) from ^{75}Se-labeled IDL-apoB to ^{75}Se-labeled LDL-apoB (kinetically determined) (Table 2). The amino acid-derived production of LDL-apoB represents that proportion of LDL-apoB turnover which cannot be accounted for by the IDL-derived LDL-apoB production (Table 3).

RESULTS

Population characteristics (Table 1)

The mean plasma LDL-apoB concentration in the six heterozygous FH subjects was 126 ± 13 mg/dl, consistent with the characteristic elevation of plasma LDL in FH in relation to the normal levels of 48 ± 6 mg/dl (1). The potential precursor, IDL apoB, had a concentration of 23 ± 11 mg/dl in these FH subjects. Plasma total cholesterol concentration averaged 382 ± 40 mg/dl, while triglyceride concentration ranged from 56 to 194 mg/dl.

The disappearance of ^{75}Se-AA from the plasma and appearance in the apoB of IDL and of LDL in the 10 days of observation was evaluated in all six patients; a representative set of data is shown in Fig. 1. The availability of ^{75}Se as the free amino acid and the ^{75}Se incorporated into ^{75}Se-labeled IDL-apoB is sufficient for analysis of their respective precursor roles of amino acids and IDL-apoB in the production of LDL-apoB.

Tracer solution (Table 2)

The kinetic parameters and their standard errors resolved in the solution of the isotopic data are given in Table 2. The mean fractional conversion of IDL to LDL was 0.0433 ± 0.0283 hr^{-1}, with an average individual standard error of the estimate for β of ±0.0121 hr^{-1}. The conversion of AA to LDL averaged 0.0045 ± 0.0025 hr^{-1}, with a mean individual standard error of the estimate for α of ±0.0010 hr^{-1}. As tabulated in Table 2, the normalized fractional residual for the least squares solution of the experimental LDL-apoB data averaged 20 ± 6%.

Steady-state solution (Table 3)

Total LDL-apoB production averaged 15.0 ± 1.5 mg/kg per day in the six heterozygous FH patients as shown in Table 3. The production from IDL catabolism was 7.1 ± 1.0 mg/kg per day, with 7.9 ± 0.9 mg/kg per day derived from free amino acid representing direct LDL-apoB synthesis.

Confirmation of methodology in normal man

The participation of IDL as the intermediate in the conversion of VLDL to LDL in normal man has been

TABLE 1. Clinical data of patients

Patient #	Age	Sex	Weight	Height	Chol.	TG	Diet	Comments
	yr		*kg*	*M*	*mg/dl*	*mg/dl*		
Familial hypercholesterolemic subjects								
LE	40	F	53	1.60	386	194	Low cholesterol, high poly unsat.	Affected first degree relative
SE	46	F	51	1.60	407	56	Low cholesterol, high poly unsat.	Affected first degree relative
HO	24	F	72	1.75	395	70	Low cholesterol, high poly unsat.	Affected first degree relative
WA	37	M	75	1.77	325	70	Low cholesterol, high poly unsat.	Affected first degree relative
HT	40	M	79	1.72	340	189	Regular diet	Affected first degree relative
BE	45	M	78	1.80	442	191	Low cholesterol, high poly unsat.	Affected first degree relative
Normal volunteer subjects								
NI	53	F	72	1.69	214	132	"Normal"	No medications
MC	54	F	65	1.58	180	41	"Normal"	No medications
MI	52	F	54	1.67	210	52	"Normal"	No medications

established by published isotopic studies which report that this pathway accounts for 80–100% of LDL-apoB production (9, 14, 15). To verify that the present ^{75}Se methodology with an analytical solution of the standard two-compartment model for LDL-apoB agrees with published studies, three normal subjects were examined. The model parameters used for LDL-apoB removal were those published by Bilheimer et al. (1) which report the average FCR in normal subjects to be 0.451 days^{-1} with a 60% intravascular distribution. The rate of transport from the intravascular to the extravascular pool used in this analysis of normal kinetic behavior is that reported by Packard et al. (3) of 0.008 hr^{-1}.

The fractional conversion of IDL to LDL was several times greater than that observed in the heterozygous FH patient, averaging 0.1910 ± 0.047 hr^{-1}, with a mean in-

TABLE 2. Kinetic parameters of low density ^{75}Se-labeled lipoprotein-apoB production

Patient #	Fractional Conversion from IDL-apoB (hr^{-1})		Fraction Conversion from Amino Acid (hr^{-1})		Average Residual Error of ^{75}Se-LDL-apoB Data Solution[a] (percent)
	β	Standard Error	α	Standard Error	
Familial hypercholesterolemic subjects					
1	0.0758	0.0121	0.0077	0.0017	19
2	0.0211	0.0017	0.0018	0.0003	15
3	0.0823	0.0341	0.0038	0.0021	28
4	0.0178	0.0140	0.0071	0.0014	26
5	0.0311	0.0044	0.0049	0.0006	15
6	0.0319	0.0062	0.0019	0.0002	19
Mean ± SD	0.0433 ± 0.0283	0.0121 ± 0.0117	0.0045 ± 0.0025	0.0010 ± 0.0008	20 ± 6
Normal volunteer subjects					
1	0.1396	0.0551	0.0100	0.0020	23
2	0.2319	0.0343	0.0043	0.0010	11
3	0.2015	0.0967	0.0108	0.0018	19
Mean ± SD	0.1910 ± 0.0470	0.0620 ± 0.0318	0.0084 ± 0.0035	0.0016 ± 0.0005	18 ± 6

[a] Normalized residual error expressed as a percent of the ^{75}Se-labeled LDL-apoB data.

TABLE 3. Low density lipoprotein-apoB steady state turnover

Patient #	Total production	IDL Derived LDL	Non-IDL Derived LDL	Plasma LDL-apoB Conc.	Plasma IDL-apoB Conc.
		mg/kg/day			*mg/dl*
Familial hypercholesterolemic subjects					
1	16.6	7.6	9.0	140	10
2	16.5	8.0	8.5	139	38
3	15.1	6.5	8.6	127	8
4	12.0	5.2	6.8	101	31
5	15.2	7.8	7.4	128	25
6	14.4	7.5	6.9	121	24
Mean ± SD	15.0 ± 1.5	7.1 ± 1.0	7.9 ± 0.9	126 ± 13	23 ± 11
Normal volunteer subjects					
1	8.3	8.0	0.3	45	6
2	9.4	6.8	2.6	52	3
3	8.8	8.0	0.8	47	4
Mean ± SD	8.8 ± 0.5	7.6 ± 0.7	1.2 ± 1.2	45 ± 9	4 ± 1

dividual standard error of the estimate for β of ± 0.0620 hr^{-1}. ^{75}Se-labeled IDL-apoB thus represents the dominant precursor for ^{75}Se-labeled LDL-apoB in these normal subjects. The normalized fractional residual for the least squares solution of the experimental ^{75}Se-labeled LDL-apoB data averaged 18 ± 6%.

Total LDL-apoB production averaged 8.8 ± 0.5 mg/kg per day, which is approximately half of that observed in the heterozygous FH patients. IDL-derived LDL-apoB production accounted for 7.6 ± 0.7 mg/kg per day, or 86% of the total LDL-apoB production. Direct LDL-apoB synthesis represented less than 14% of the total production, at 1.2 ± 1.2 mg/kg per day. These data confirm the role of IDL in normal man as the virtually exclusive precursor of LDL-apoB as previously suggested by other investigators using different methodologies (9, 14, 15).

DISCUSSION

The present studies indicate two pathways of production of LDL-apoB in patients with heterozygous familial hypercholesterolemia, and suggest a metabolic basis for the structural heterogeneity of LDL in man (10–12). Such a conclusion is similar to that of Fidge and Poulis (25) who investigated LDL synthesis from a labeled amino acid in the rat. These investigators observed that the major component of LDL (d 1.040–1.63 g/ml, S_f 0–5) accounts for 65% of the circulating LDL and is produced independently of VLDL or IDL catabolism. Illingworth (26) similarly reported that up to 15% of the LDL produced in the squirrel monkey was secreted directly into the plasma on the basis of tracer amino acid injection studies. More recently, Sparks and Marsh (27) have reported metabolic heterogeneity of LDL-apoB removal by the liver in the rat. Their data suggest that a subclass of apoB within LDL may be metabolized by either hepatic clearance of extrahepatic pathways of removal depending upon the structure of the LDL particles.

Previous investigations in man have reported that VLDL is a metabolic precursor of LDL (13–16). Based upon the similarity of total VLDL-apoB turnover and LDL-apoB turnover, it has been suggested that the VLDL-apoB production is sufficient to account for all LDL-apoB production (14). However, the observations from several different analyses (15, 16, 28, 29) have shown that a significant portion of VLDL-apoB turnover is directed toward pathways other than LDL production. Thus, alternative pathways of LDL-apoB synthesis must be postulated.

Consistent with this possibility, studies with simultaneous infusions of labeled VLDL and LDL in homozygous FH have demonstrated that a marked discrepancy exists between VLDL-apoB turnover and LDL-apoB turnover, suggesting that alternative routes of LDL-apoB synthesis are present in FH (5). Double labeled studies in heterozygous FH have reported that non-VLDL-derived LDL production may account for 50% of LDL-apoB production (9).

Previous studies have emphasized the obligatory role of IDL as an intermediate in the conversion of VLDL to LDL (9, 15, 28, 29). Direct hepatic production of IDL-apoB could account for the discrepancy between VLDL and LDL-apoB turnover in FH. Alternatively, direct synthesis of LDL-apoB independent of either VLDL of IDL would also explain the reported data.

Such an alternative pathway might also result in an LDL species of abnormal lipid and/or protein composition, leading to the structural heterogeneity of LDL observed in man. Our data now demonstrate that 54 ± 6% of LDL-apoB in heterozygous FH is derived fron non-IDL precursors. This conclusion is similar to that of Janus et al. (9) who observed a range from 20–72% in their six heterozygous FH subjects. In our analytical method of determining flow through the two pathways, no assumptions relative to a compartmental model of AA or of IDL-apoB behavior have been made. Instead, the discrete data at each timepoint for ^{75}Se-AA and ^{75}Se-labeled IDL-apoB have been utilized.

Several lines of evidence suggest the potential for direct synthesis of LDL-apoB by some pathway other than VLDL or IDL catabolism. In nonhuman primates, the liver may be stimulated to directly secrete cholesterol-rich particles within the LDL density range (26). Similarly, in the rat, direct hepatic synthesis of LDL-apoB has been demonstrated (25). Recently, studies using equilibrium density gradient ultracentrifugation of serum LDL from normal human subjects have indicated structural heterogeneity of LDL consistent with differences in both size and cholesterol-triglyceride composition of subspecies of the lipoprotein (10, 11). Differences in metabolic interrelationships of the larger and smaller fractions may be implicated from these observations, as well as the possibility of differences in the pathways for production of larger and smaller LDL. The alternative pathway for LDL synthesis shown in our data might result in an LDL species of abnormal lipid composition, leading to the potential for increased molecular heterogeneity. A similar event may occur in normal man under the influence of increased consumption of dietary cholesterol as observed by Mistry et al. (30). These authors reported an increase in cholesterol-rich LDL concentration in these subjects, without an increase in VLDL level. Though a kinetic evaluation of lipoprotein interrelationships was not performed, the data were interpreted by the authors as consistent with stimulation of the liver to directly secrete cholesterol-rich particles within the LDL density range.

The data from our six heterozygous FH patients support the concept that direct synthesis of LDL-apoB independent of IDL catabolism constitutes a significant kinetic characteristic of this genetic disease.

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