Overproduction of a kinetic subclass of VLDL-apoB, and direct catabolism of VLDL-apoB in human endogenous hypertriglyceridemia: an analytical model solution of tracer data

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Abstract To investigate the participation of the major apoprotein involved in triglyceride transport in the pathogenesis of endogenous hypertriglyceridemia, five kinetic studies of apoprotein B were conducted in volunteer normolipidemic subjects and six studies in four patients with endogenous hypertriglyceridemia. The transport of apoprotein B within four kinetic subclasses of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) was studied by injection of [^{75}Se]selenomethionine. A 24-fold increase in the entry of newly synthesized apoprotein B at the initial kinetic subclass of the four-compartment VLDL delipidation sequence characterized the hypertriglyceridemic studies relative to normal subjects. Moreover, approximately 75 mg/kg per day of VLDL-B turnover reflected direct catabolism independent of conversion to IDL and/or to LDL, in contrast to the 8 mg/kg per day observed in controls. IDL-B was derived from VLDL-B in both normal and hypertriglyceridemic subjects, and was responsible for greater than 70% of all LDL-B synthesis. LDL-B pool size and turnover were indistinguishable in hypertriglyceridemic subjects from that observed in normal subjects. These studies suggest that two kinetic phenomena may characterize the pathophysiology of endogenous hypertriglyceridemia: *a*) overproduction of apoB within a kinetic subclass of VLDL and *b*) preferential catabolism of hypertriglyceridemic VLDL without prior conversion to IDL/LDL.—**Eaton, R. P., R. C. Allen, and D. S. Schade.** Overproduction of a kinetic subclass of VLDL-apoB, and direct catabolism of VLDL-apoB in human endogenous hypertriglyceridemia: an analytical model solution of tracer data. *J. Lipid Res.* 1983. **24:** 1291–1303.

Supplementary key words VLDL • IDL • LDL • apoprotein B • endogenous hypertriglyceridemia • [^{75}Se]selenomethionine

Normal synthesis and/or secretion of VLDL depends upon the presence of apoprotein B (apoB), the major transport protein involved in triglyceride (TG) metabolism. Newly secreted apoB-VLDL is converted in turn to IDL and LDL, providing the normal metabolic sequence of apoB metabolism. In hypertriglyceridemic states, augmented production of VLDL and/or production of an abnormal "hypertriglyceridemic VLDL" may occur (1–3). Such "hypertriglyceridemic VLDL" may be *a*) the consequence of increased production of TG in the presence of normal apoB secretion as suggested in carbohydrate-induced hypertriglyceridemia (4); *b*) secondary to increased production of both TG and apoB as proposed in familial hypertriglyceridemia (5); or *c*) due to overproduction of apoB-VLDL alone as reported in familial combined hyperlipidemia (4, 5). From a kinetic standpoint, the augmented secretion of apoB-VLDL may represent production of an abnormal subclass of TG-enriched VLDL, which could be the product of any or all of these events leading to "hypertriglyceridemic VLDL" (3).

In addition to the proposed overproduction of an apoB-containing subclass of VLDL in endogenous hypertriglyceridemia, several lines of investigation suggest that VLDL from hypertriglyceridemic subjects may be catabolized by pathways other than the normal metabolic sequence of VLDL. As recently reviewed, hypertriglyceridemic VLDL but not normal VLDL will: *a*) suppress the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in cultured human fibroblasts and endothelial cells; *b*) produce triglyceride accumulation in macrophages; and *c*) be bound, internalized, and degraded via the LDL receptor pathway by cultured human fibroblasts and by human mononuclear cells (2, 6). Each of these events may represent direct catabolism of hypertriglyceridemic VLDL, presumably by LDL receptor interaction, that is minimally observed with VLDL from normal subjects.

Abbreviations: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; PAGE, polyacrylamide gel electrophoresis.

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To examine these hypotheses in vivo, we have utilized [^{75}Se]selenomethionine as an endogenous tracer for amino acid incorporation into newly synthesized apoB within VLDL, IDL, and LDL in normal and hypertriglyceridemic subjects. To address both the metabolic synthesis of VLDL subfractions as well as catabolic pathways of VLDL metabolism, our analysis utilized the detailed kinetic model concept proposed by Berman et al. (7), in which a four-compartment subclass system defines VLDL metabolism prior to conversion to IDL. The details and description of the model adapted for endogenous tracer investigation of apoB metabolism have been previously reported (8). In this system, the production of apoB into the individual lipoprotein subfractions and the catabolic events describing the fate of apoB within each lipoprotein species are defined. The recent use of tritiated leucine as an endogenous tracer of lipoprotein metabolism in a similar kinetic model has been reported by Fisher et al. (3). Our data support the concept that overproduction of apoB within a kinetic subfraction of VLDL is characteristic of these hypertriglyceridemic patients, and demonstrate quantitatively increased direct catabolism of both VLDL and IDL independent of conversion to LDL.

Methods

Eleven studies were carried out in normal volunteer and hypertriglyceridemic subjects described in **Table 1.** For the hypertriglyceridemic subjects, insufficient family members were available to allow conclusions regarding inheritance of a specific form of hyperlipoproteinemia. Body adiposity as evaluated by the Ponderal Index of Livi (9) was between lean (<22) and obese (>25) in four of the five controls, and in three of the four hyperlipidemic subjects.

Subject data

Hypertriglyceridemic subject No. 6 was a 40-year-old male with a history of coronary heart disease including myocardial infarction noted at age 31 years, with coronary bypass surgery performed at age 39 years. He had a history of smoking (65 packs/year), and a 20-year history of alcohol abuse allegedly corrected. At the time of original evaluation his fasting plasma triglyceride concentration was "greater than 1,000 mg/dl" and his cholesterol concentration was 466 mg/dl, with a normal insulin and glucose pattern on oral glucose tolerance testing. Following caloric restriction, his plasma triglyceride concentration fell to 363 mg/dl and his cholesterol level fell to 320 mg/dl. No palmar or tendon xanthoma were present. He refused drug therapy for his hyperlipemia, and was subsequently lost to follow-up. No family history of hyperlipidemia could be obtained. A phenotypic diagnosis of Type IIB hyperlipemia was entertained complicated by alcohol abuse and smoking, consistent with the syndrome of "combined hyperlipoproteinemia".

Hypertriglyceridemic subject No. 7 was a 54-year-old male with the diagnosis of Type II diabetes made at age 40. He was treated with oral sulfonylurea therapy until insulin was initiated at age 52 years. Hyperlipemia was first diagnosed at age 52 years with a fasting plasma

TABLE 1. Clinical data

Patient No.	Phenotype	Age	Sex	Weight	Height	Ponderal Index[a]	Chol	TG	Comments
		yr		*kg*	*m*		*mg/100 ml*		
Normolipidemic subjects									
1	Normal	54	M	84	1.83	23.5	216	120	
2	Normal	33	F	53	1.58	23.4	161	57	
3	Normal	52	F	54	1.67	22.3	210	52	
4	Normal	54	F	65	1.58	25.4	180	41	
5	Normal	53	F	72	1.69	24.6	214	132	
Hypertriglyceridemic subjects									
6	IIb	40	M	79	1.72	24.9	340	453	
7	IV	54	M	102	1.87	24.9	312	651	
8[b]	III	46	F	78	1.64	26.0	344	372	Tendon xanthoma and
9[b]		45	F	72	1.59	26.1	473	472	palmar xanthoma
10[c]	III	29	M	87	1.83	24.2	478	441	Palmar xanthoma and
11[c]		30	M	84	1.83	23.9	378	630	tendon xanthoma

[a] Ponderal Index of Livi (9) = (10 (BW) 1/3)/Ht, when lean < 22, and obese > 25.
[b] Same subjects studied two times under the same conditions a year apart.
[c] Same subject studied two times under the same conditions a year apart.

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triglyceride concentration of 2,340 mg/dl, and a cholesterol concentration of 405 mg/dl. With dietary management of caloric intake and carbohydrate and fat limitation, his plasma triglyceride levels approached 650 mg/dl, while his cholesterol level was reduced to the 230 mg/dl range. Plasma lipoprotein electrophoresis demonstrated a marked pre-beta band consistent with Type IV hyperlipoproteinemia. No palmar or tendon xanthoma were present. At the time of investigation, the patient was under therapy with insulin, and had achieved adequate glucose control.

Hypertriglyceridemic subject No. 8, 9 was a 46-year-old female with a history of tuberous xanthoma over the elbows since age 38 years. At age 40 she was diagnosed as having lymphocytic thyroiditis; she was treated with a subtotal thyroidectomy with thyroid hormone replacement. At the time of initial evaluation at the University of New Mexico hospital, she was clinically hypothyroid, with a TSH elevated to 68 μU/ml, and fasting plasma triglyceride concentration of 1,000 mg/dl and a cholesterol concentration of 900 mg/dl. Following appropriate L-thyroxine replacement to a normal plasma TSH level, her plasma cholesterol was reduced to levels in the 450 mg/dl range, with plasma triglyceride concentration in the 350 mg/dl range. Yellow-orange plaques were observed in the creases of her hands, with yellow xanthoma on her finger pads, buttocks, and legs. Lipoprotein electrophoresis at this time demonstrated a "broad beta band extending through the pre-beta position". For the next 2 years the patient was additionally treated with clofibrate, and dietary restriction of cholesterol and saturated fatty acids. A marked resolution of her palmar xanthoma and soft-tissue xanthoma was observed, though her plasma lipid levels remained in the range of 350 mg/dl for cholesterol and 450 mg/dl for triglyceride. Clinical and laboratory criteria at the time of investigation were compatible with Type III hyperlipoproteinemia aggravated by concurrent hypothyroidism. Family investigation by letter failed to identify similar clinical problems. No history of alcohol ingestion, and no evidence of diabetes was observed. At the time of investigation, the patient was receiving thyroid therapy and had achieved an euthyroid state.

Hypertriglyceridemic subject No. 10, 11 was a 30-year-old male with hyperlipemia first diagnosed at age 26 when he presented with painful yellow xanthoma on his palms and elbows, and plasma triglyceride levels of 800 mg/dl and cholesterol levels of 450 mg/dl. He was treated with carbohydrate restriction, weight reduction, and clofibrate. There was no positive family history for hyperlipemia, and the patient had no alcoholic history. In response to clofibrate therapy, a reduction of plasma triglyceride to 398 mg/dl and cholesterol to 283 mg/dl was achieved, though this response was evanescent over the years of follow-up. The persistent pre-beta band on lipoprotein electrophoresis was consistent with Type III hyperlipemia. No abnormalities of thyroid function and no carbohydrate intolerance were noted, though basal insulin levels were elevated suggesting some degree of peripheral insulin resistance.

Clinical protocol

All subjects were hospitalized in the Clinical Research Unit and received a standard isocaloric diet with an intake of 38% carbohydrate, 45% fat, and 17% protein. Informed consent was obtained prior to initiation of studies, and approval was received by the University of New Mexico Human Research Review Committee. The subjects were fasted for 16 hr prior to the injection of labeled amino acid 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 5–10 days (8). All samples were obtained using 0.1% EDTA as the anticoagulant, with immediate addition of glutathione to 0.02% to prevent lipid peroxidation as described by Lee (10). Disappearance from the plasma of ^{75}Se as the free amino acid was monitored, and incorporation into the apoB polypeptide of VLDL, IDL, and LDL was evaluated either by Sephadex G-150 gel filtration (11–14) or by quantitative polyacrylamide gel electrophoresis (PAGE) isolation of the apoprotein from the lipoprotein fractions separated by serial ultracentrifugation (15). Studies 1, 8, and 9 utilized gel filtration separation, while studies 2–7, and 10, 11 utilized PAGE separation.

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, as 12°C, in a Beckman Model A ultracentrifuge (16). Eight ml of

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

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plasma from each sample was divided and placed in two 7-ml ultracentrifuge tubes and overlayered with 3 ml of normal saline containing 0.05% EDTA. The very low density lipoproteins were separated in the d < 1.006 g/ml fraction of serum after centrifugation for 22 hr at 105,000 *g*. 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 volumes of KBr solution of d 1.063 g/ml, and re-centrifuged for 22 hr to isolate the LDL fraction. In all samples from hyperlipemic patients, the isolated VLDL, IDL, and LDL were re-centrifuged at their respective densities with appropriate solution overlay to assure a washed isolation of each lipoprotein species. The lipoproteins were dialyzed for 72 hr against 100 volumes of 0.01% EDTA solution (pH 7.0) at 4°C; the bath was changed every 12 hr. Total radioactivity present in each lipoprotein fraction at this point was utilized to monitor for losses and recovery at each subsequent isolation and purification step. Total protein content within each lipoprotein fraction was determined by the method of Lowry et al. (17) using bovine albumin as the standard. Any turbidity due to lipid that appeared after color development was removed by extraction with water-saturated ether before determination of absorbance (18). The concentrated lipoprotein fractions were free of albumin, as monitored by immunodiffusion tests.

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 (19) to prevent potential losses of protein into organic extraction solutions (20, 21). Extractions were performed on lyophylized lipoprotein fractions with anhydrous solvents, as previously described (12). Aliquots of dialyzed VLDL, IDL, and LDL were lyophilized and delipidated at 4°C with an initial extraction of 50 ml of absolute ethanol–diethyl ether 3:1 (v/v), for 16 hr. Following centrifugation at 2,500 rpm, the solvent was decanted and saved for monitoring protein losses, and the aggregated protein was dispersed into two similar extractions carried out for 4 hr each. The protein was then rinsed twice with 50 ml of diethyl ether at 4°C for 2 hr each. The ether was removed by centrifugation, and the apolipoproteins were dried to a constant weight over P_2O_5 in a vacuum desiccator. The protein was completely soluble to a concentration of 20 mg/ml in 0.2 M Tris-HCl buffer, pH 8.2, containing 0.10 M sodium decyl sulfate. Samples containing 1 mg of protein were chromatographed on thin-layer silica gel G plates with petroleum ether–diethyl ether–acetic acid 90:10:1 (22); spots were visualized with iodine vapor and anisaldehyde. No triglyceride or cholesterol was detected by this method.

To monitor the potential loss of significant amounts of apolipoproteins into the organic phases, the ethanol–diethyl ether phases and the diethyl ether phases were separately evaporated to dryness in a rotary evaporator. No radioactivity in excess of background could be detected in the residue from organic extraction from the lipoprotein preparations by use of a Nuclear Chicago Autogamma counter with triplicate 10-min counting of each sample. After determination of potential ^{75}Se-radioactivity within each sample, the residues were extracted with chloroform–methanol 3:1 (v/v) and solubilized in 0.2 M Tris-HCl buffer, pH 8.2, containing 0.10 M sodium decyl sulfate. No protein was detected by the Lowry reaction, and no protein-staining bands were observed by disk-gel electrophoresis (see method below) of samples from either VLDL, IDL, or LDL extractions. These results are consistent with the data of Scanu and Edelstein (21), who also reported that extraction of lyophilized VLDL with absolute ethanol–diethyl ether 3:1(v/v) did not remove detectable apoprotein. These investigators also noted that a single extraction with absolute ethanol–diethyl ether 3:1 failed to completely remove free and esterified cholesterol and triglycerides, hence the necessity for the repeated extractions and the ether extractions utilized in our studies. While the method utilized in our studies does not exclude the possibility of loss of a peptide in concentrations below detection by our method, it does provide a reproducible preparation with which to determine the specificity of ^{75}Se incorporation into human apoprotein.

Gel filtration

Sephadex superfine G-150 in 0.2 M Tris-HCl, pH 8.2, was packed in columns (115 × 1.2 cm), and 1–10 mg of solubilized protein was applied in a volume of 250 µl or less, as described by Eisenberg et al. (23). The columns were eluted with 0.2 M Tris-HCl buffer containing 0.002 M decyl sulfate, and 1.0-ml fractions were collected.

Quantitative polyacrylamide gel electrophoresis

The apoB moiety of the apoprotein of VLDL, 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

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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. Correction for decay of ^{75}Se and loss of radioactivity by isolation of apoproteins was performed to account for the total cpm found in each lipoprotein fraction following the dialysis which removed free ^{75}Se-labeled amino acid. The labeled apoB was evaluated in the initial 3-mm slice termed zone 1 by Bilheimer, Eisenberg, and Levy (24), as previously utilized by Berman et al. (7) in their tracer studies.

To quantitate the apoB concentration and plasma pool for VLDL-B, IDL-B, and LDL-B, analytical gel electrophoresis was performed as described above, utilizing 10 μg of apoprotein for each lipoprotein fraction 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 (25). Parallel gel electrophoresis of bovine albumin (Sigma) was used as the standard, with protein recovery averaging 92 ± 4%. This approach permitted quantitation of both the protein and tracer content of the same isolated peptide from the gel, which is optimal for kinetic analysis of apoB metabolism.

The ^{75}Se radioactivity incorporated into apoB isolated from VLDL, IDL, and LDL is expressed as cpm/ml of plasma for each 8-ml plasma sample. The pattern of incorporation of ^{75}Se-labeled amino acid into VLDL-apoB, IDL-apoB, and LDL-apoB is graphically depicted in **Fig. 1** for a representative normal subject (No. 5) and in **Fig. 2** for a representative hypertriglyceridemic subject (No. 8). The profiles in incorporation into apoB in the three lipoproteins species is similar to that reported by Stahelin (26) for ^{75}Se-labeled amino acid incorporation into the total apoprotein moiety of these lipoprotein fractions. A similar sequence of incorporation from ^{3}H-labeled amino acid injection (leucine) into hyperlipemic subjects has also been reported by Fisher et al. (3) and Phair et al. (13) who monitored apoB isolated by gel filtration chromatography. As can be appreciated in Figs. 1 and 2, the hypertriglyceridemic patient demonstrated a greater accumulation of ^{75}Se within VLDL and IDL relative to LDL, than is seen in these lipoproteins obtained from normal subjects (Fig. 1).

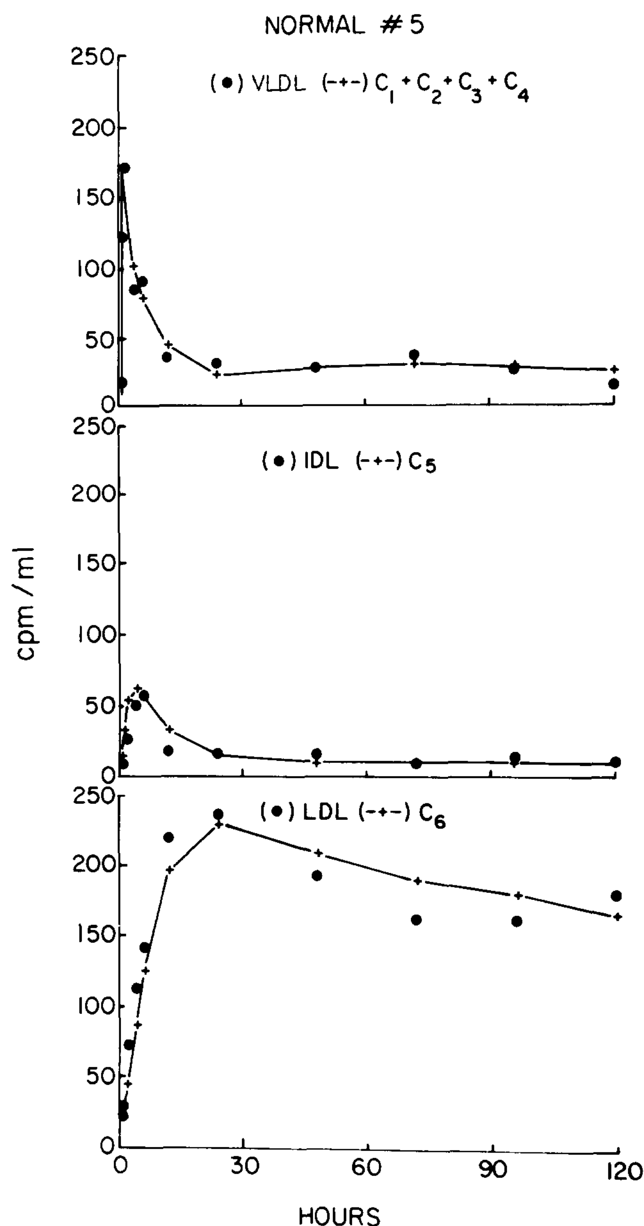

Fig. 1. Entry of ^{75}Se-labeled amino acid into beta-apoprotein within plasma VLDL, IDL and LDL in a representative normal subject No. 5. An injection of [^{75}Se]selenomethionine was given at time zero, and the incorporation data was expressed as cpm/ml of plasma from which the lipoproteins were extracted. Observed data = ●; model-generated data = — + — for the indicated compartments.

Beta-apoprotein model

The data were fitted to the previously described seven-compartment model of apoB (8) using the interactive computer program KABIS (27) to determine the metabolic parameters characteristic of each subject. This model, shown in **Fig. 3**, has been adapted from that of Berman et al. (7) for the analysis of in vitro labeled apoprotein injection studies. Adaptation for tracer investigation of new protein synthesis from amino acid precursor requires the capability of input of ^{75}Se-labeled amino acid directly into the apoB of VLDL, IDL, and LDL compartments. As previously described (8), this precursor function is generated by a three ex-

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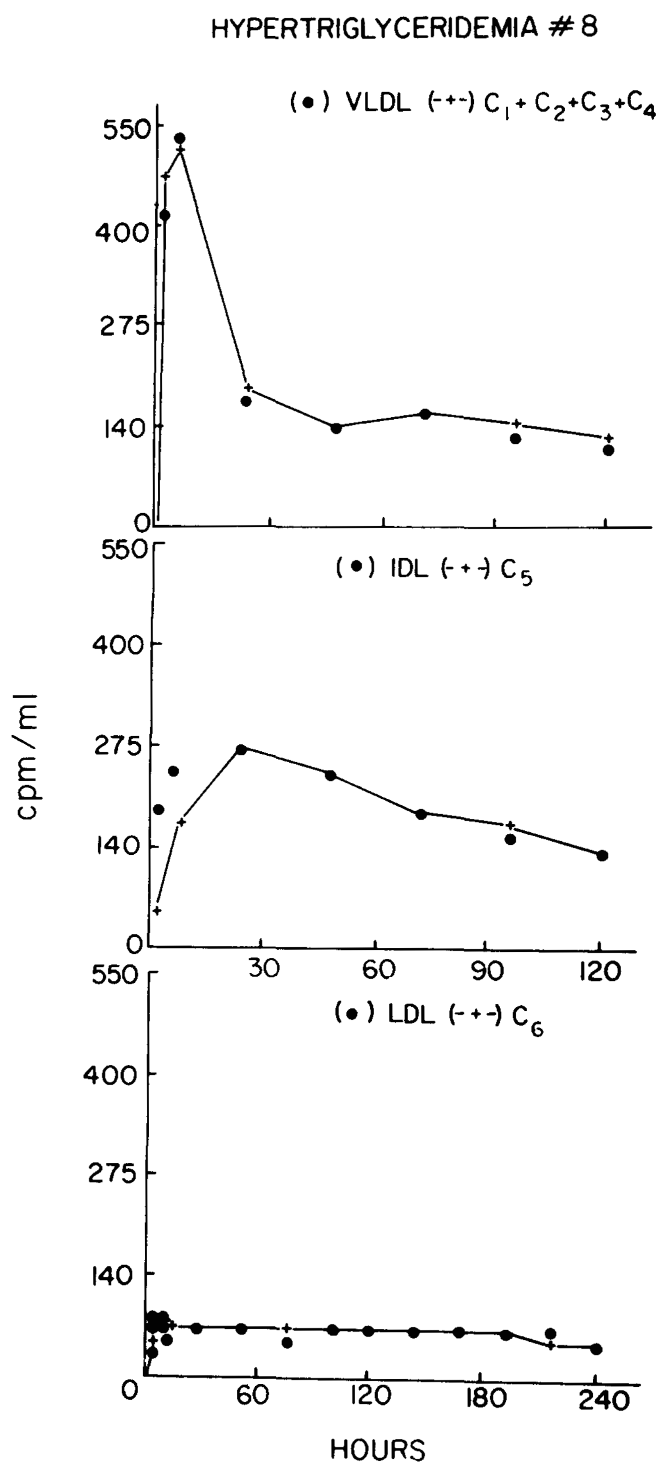

Fig. 2. Entry of ^{75}Se-labeled amino acid into beta-apoprotein within plasma VLDL, IDL, and LDL in a representative hypertriglyceridemic subject No. 8. An injection of [^{75}Se]selenomethionine was given at time zero, and the incorporation data was expressed as cpm/ml of plasma from which the lipoproteins were extracted. Observed data = ●; model-generated data = — + — for the indicated compartments.

ponential sum which matches the experimental data for plasma amino acid specific activity. This function provides a continuous input of ^{75}Se-labeled amino acid into lipoprotein metabolism which includes recycling ^{75}Se-labeled amino acid back into the precursor system implied by the "tail" of the actual ^{75}Se-labeled amino acid curve (8), as similarly reported by Fisher et al. (3) using [^{3}H]leucine as an endogenous tracer.

The Berman model identifies a delipidation sequence of four kinetic subclasses for VLDL metabolism, entering the IDL compartment (7). Newly synthesized apoB may enter the VLDL system via the initial subclass and undergo a relatively prolonged transit through four sequential compartments of the step-wise delipidating pathway. It may also enter the terminal subclass (fourth VLDL compartment) in a presumably TG-depleted state, and rapidly be removed from the plasma or be converted to IDL. Entry of tracer into the initial VLDL compartment-1 provides kinetic characteristics similar to that achieved with a separate β-VLDL compartment of reduced turnover rate as utilized by Berman et al. (7). However, our data do not justify this additional complexity, so we have not used a β-VLDL compartment as previously discussed (8). This system of VLDL catabolism is similar to that reported by Fisher et al.

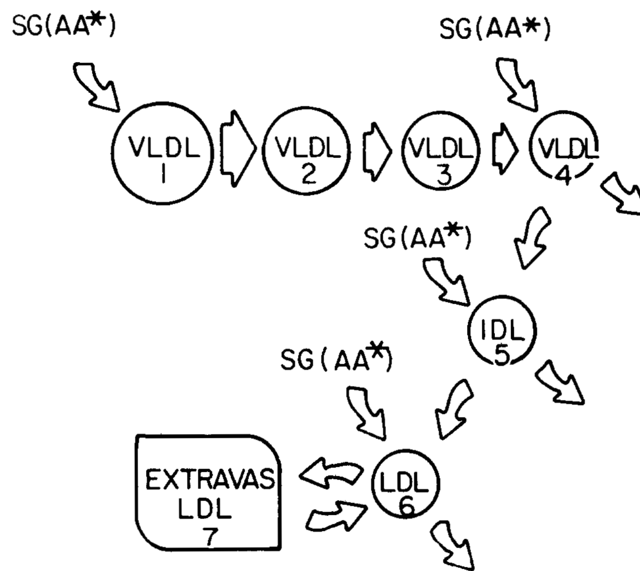

Fig. 3. Schematic compartmental model of beta-apoprotein metabolism in man following the injection of an amino acid tracer for protein synthesis ([^{75}Se]selenomethionine) as previously reported (8). The model includes a four-compartment delipidation sequence of VLDL with potential sites of synthesis at either the proximal or terminal (4th) VLDL subclass; a single compartment of IDL; and a single compartment of LDL with extravascular recycling. Independent synthesis may occur at the multiple sites indicated, and the catabolic losses may occur from VLDL, IDL, or LDL.

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TABLE 2. Kinetic parameters of ^{75}Se-VLDL beta-apoprotein studies

Study	Lipoprotein Phenotype	VLDL-B Data						
		Pool	Concentration	Turnover Rate	Turnover	FCR[a] Day^{-1}	Proximal[b] Entry	Direct Catabolism[c]
		mg	*mg/100 ml*	*mg/kg per day*	*mg/day*		*mg/kg per day*	
Normolipidemic subjects								
1	N	164	4.7	29	2,478	15.1	0	20
2	N	50	2.3	14	752	15.0	0	5
3	N	32	1.5	9	483	15.1	1.3	3
4	N	78	2.9	16	1,073	13.7	0.5	0
5	N	140	4.7	21	1,483	10.6	2.9	12
Mean ± SD		93 ± 57	3.2 ± 1.4	18 ± 8	1,254 ± 779	13.9 ± 1.9	1.2 ± 1.3	8 ± 8
Hypertriglyceridemic patients								
6	IIb	414	12.7	44	3,500	8.4	11.5	0
7	IV	890	21.1	87	8,864	9.9	14.8	76
8	III	921	28.6	85	6,669	7.2	30.8	72
9	III	363	12.2	54	3,874	10.6	7.5	43
10	III	1,028	28.5	125	10,875	10.5	17.5	65
11	III	2,318	66.7	250	21,034	9.1	55.0	190
Mean ± SD		989 ± 708	28.3 ± 20.1	107 ± 75	9,136 ± 6,484	9.3 ± 1.3	22.8 ± 17.6	75 ± 63

[a] FCR represents the fraction of the intravascular pool metabolized each day.
[b] Newly synthesized apoB entering the VLDL delipidation pathway via the first compartment instead of the terminal (fourth) compartment (see Fig. 3).
[c] Direct catabolism does not convert to LDL-beta-apoprotein.

(3) and Phair et al. (13) who also did not include a β-VLDL pool in their studies utilizing tracer leucine injection with endogenous incorporation into apoB. The IDL-B is derived from VLDL-B or by direct synthesis from the amino acid precursor pool. As previously reported, the LDL system requires an extravascular recycling tissue reservoir resulting in a two-compartment system (7, 8, 13, 15).

The rate constants used to fit the data for each patient are tabulated in the Appendix. The precision of each parameter for a given patient solution is defined as ± parameter deviation which results in a 1% change in the residual of the fit of the data. The steady-state solution of the model is calculated with the plasma VLDL-B, IDL-B, and LDL-apoB pool as the additional information needed to permit the transformation from the isotopic kinetic solution.[2] In determining apoB production for each patient, the calculated plasma volume (41.3 ml/kg BS) (28) was used as the intravascular space.

[2] The calculation of steady-state relationships from tracer analysis in any experimental analysis requires knowledge of either stable material synthesis (frequently obtained from urine excretion) or a minimum of one component of plasma stable material concentration in kinetic linkage with the system. Urinary excretion data can only be applied when a single labeled apoprotein is injected into the vascular space.

All numerical calculations were performed on an IBM-3032 digital computer.

RESULTS

ApoB metabolism in VLDL-B (Table 2)

Normolipidemic subjects. As shown in Table 2, the beta-apoprotein content of VLDL ranged from 1.5 to 4.7 mg/dl and the plasma pool averaged 93 ± 57 mg in these five volunteer subjects. Most of the newly synthesized VLDL-B (18 ± 8 mg/kg per day) entered the terminal subclass of VLDL, with entry into the initial subclass ranging less than 2.9 mg/kg per day.

The major removal was conversion to IDL-beta-apoprotein. While this conversion was the dominant pathway of loss from VLDL, a small amount of direct removal was observed in three of the studies and accounted for as much as 20 mg/kg per day of the catabolism in one of the subjects (mean for the group = 8 ± 8 mg/kg per day).

Hypertriglyceridemic subjects. As shown in Table 2, the beta-apoprotein content of VLDL within the hypertriglyceridemic subjects was markedly increased relative to that seen in control subjects. The range of concentration from 12.2 to 66.7 mg/dl represents a three- to eightfold expansion of the plasma pool which averaged 989 ± 708 mg in the six studies.

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In every study in hypertriglyceridemic subjects, a tenfold or greater amount of the newly synthesized VLDL-B entered the VLDL sequence via the proximal subclass of the delipidation pathway as compared with controls. Thus, of the quantitative synthesis ranging from 44 to 250 mg/kg per day, the entry into the initial kinetic subclass of VLDL-B averaged 23 mg/kg per day, which contrasts with the maximum of 2.9 mg/kg per day observed in control subjects. In addition, a fivefold increase in newly synthesized VLDL-B entered via the terminal subclass of VLDL, representing a marked increase in this normal pathway. Direct removal, exclusive of conversion to IDL, dominated VLDL degradation in five of the six studies and accounted for 75 mg/kg per day in contrast to the 8 mg/ kg per day in controls.

Apo-B metabolism in IDL-B (Table 3)

Normolipidemic subjects. As shown in Table 3, the beta-apoprotein content of plasma IDL ranged from 1.9 to 6.7 mg/100 ml and the plasma pool averaged 89 ± 57 mg in the five volunteer subjects. IDL-B functioned as a metabolic intermediate between VLDL-B and LDL-B, receiving 82–100% of its beta-apoprotein component directly from VLDL. IDL-B synthesis ranged from 6 to 20.0 mg/kg per day. In the two subjects with direct synthesis exclusive of the VLDL precursor, this route contributed a maximum of only 4 mg/kg per day. A proportion of the IDL-B removal was by direct catabolism, exclusive of conversion to LDL-B. Four of the five subjects demonstrated direct catabolism ranging from 25% to 50% of the total removal, resulting in an average of 4 ± 4 mg/kg per day.

Hypertriglyceridemic subjects. As shown in Table 3, the beta-apoprotein content of IDL within the hypertriglyceridemic subjects was variable, ranging from values similar to that seen in normals to marked elevations in concentration. In general, the plasma pool was increased and averaged 749 ± 476 mg.

The VLDL-B transport into the IDL-B pool accounted for 99% of the total IDL-B synthesis, with the total flow ranging from 8 to 60 mg/kg per day in the five subjects.

As in normals, significant loss from the IDL beta-apoprotein pool occurred by catabolic removal unrelated to conversion to LDL. In the five studies in which such catabolism was demonstrated, this averaged 48 ± 32% of the total turnover and accounted for 22 ± 21 mg/kg per day in contrast to the 4 mg/kg per day observed in controls.

The relationship between IDL-B production and concentration is graphically depicted with the VLDL-B data in **Fig. 4** for both hypertriglyceridemic and normolipidemic subjects. Up to a plasma concentration of approximately 20 mg/dl, a linear increase in production is associated with an increase in apoprotein concentration ($r^2 = 0.97$, $P < 0.001$). However, in two studies (Nos. 8, 9), it is apparent that marked elevations in IDL-B concentration are associated with relatively low levels of production. In these studies, a defect in removal is implicated in the accumulation of IDL-B in the plasma.

TABLE 3. Kinetic parameters of ^{75}Se-IDL beta-apoprotein studies

Study	Lipoprotein Phenotype	IDL-B Data						
		Pool	Concentration	Turnover Rate	Turnover	FCR Day^{-1}	From VLDL	Direct Catabolism[a]
		mg	*mg/100 ml*	*mg/kg per day*	*mg/day*		*mg/kg per day*	*mg/kg per day*
Normolipidemic subjects								
1	N	109	3.1	9	790	7.2	9	3
2	N	54	2.5	10	519	9.6	9	2
3	N	43	1.9	6	310	7.2	6	0
4	N	180	6.7	20	1,300	7.2	16	10
5	N	57	1.9	8	612	10.7	8	3
Mean ± SD		89 ± 57	3.2 ± 2.0	11 ± 5	706 ± 374	8.4 ± 1.7	10 ± 4	4 ± 4
Hypertriglyceridemic patients								
6	IIb	416	12.7	44	3,500	8.4	44	28
7	IV	137	3.2	10	989	7.2	10	2
8	III	1,504	46.6	14	1,123	0.7	13	7
9	III	1,024	34.4	8	612	0.6	8	1
10	III	719	20.0	60	5,177	7.2	60	49
11	III	695	20.0	60	5,006	7.2	60	43
Mean ± SD		749 ± 476	22.8 ± 15.5	33 ± 25	2,735 ± 2,091	5.2 ± 3.5	32 ± 25	22 ± 21

[a] Direct catabolism does not convert to LDL-beta-apoprotein.

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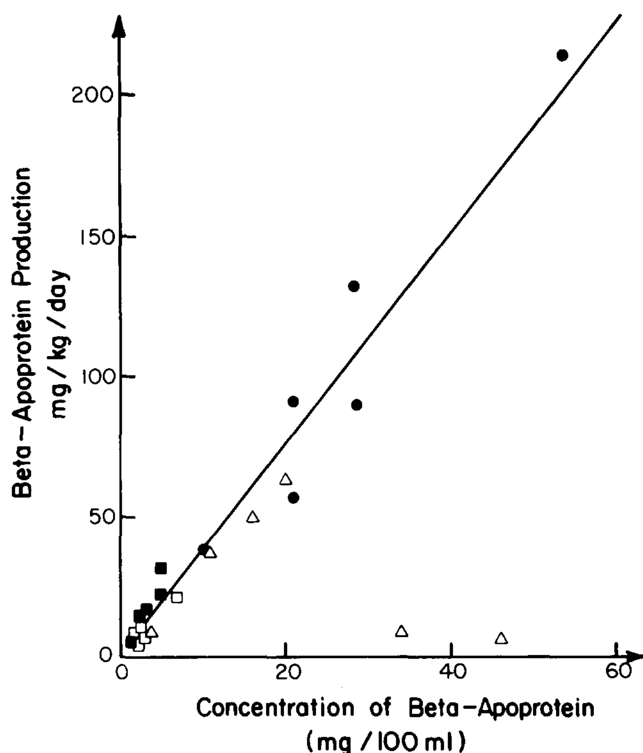

Fig. 4. Relationship between production of beta-apoprotein within VLDL or IDL versus plasma concentration of VLDL and IDL. Both normolipidemic and hypertriglyceridemic subjects are plotted ($r^2 = 0.97$, $P < 0.001$). N-VLDL = (■); TG-VLDL = (●); N-IDL = (□); TG-IDL = (△).

ApoB metabolism in LDL-B (Table 4)

Normolipidemic subjects. As shown in Table 4, the beta-apoprotein content of plasma LDL ranged from 34 to 48 mg/dl by duplicate assay, with an accuracy of ±10% in the determination. Based upon the plasma volume, the plasma pool averaged 1,119 ± 245 mg. IDL-B represented the precursor for 72 ± 15% of the LDL-B turnover, so that total LDL-B synthesis included a small amount of additional direct synthesis exclusive of the VLDL-IDL system as previously reported (15). Total LDL-B synthesis ranged from 7 to 12 mg/kg per day in these five subjects.

Hypertriglyceridemic subjects. As shown in Table 4, the concentration of beta-apoprotein within LDL was indistinguishable in hypertriglyceridemic subjects and in the normals (normal = 41 ± 5 mg/dl; hypertriglyceridemia = 52 ± 8 mg/dl). Similarly, LDL-B synthesis ranged from 8 to 16 mg/kg per day, representing a total metabolism similar to that of the normal lipidemic subjects. The intravascular distribution of LDL-beta-apoprotein tended to be reduced from the approximate 66 ± 2% observed in normals. In four of the six studies, less than 34% of the total body pool resided within the vascular compartment, while the average for the group was 40 ± 16%.

DISCUSSION

The present investigations conducted with [^{75}Se]-selenomethionine, contribute additional information to the understanding of endogenous hypertriglyceridemia by examining the in vivo production and catabolism of the two major TG-bearing lipoproteins, VLDL-B and IDL-B, and the kinetic subclasses of VLDL.

TABLE 4. Kinetic parameters of ^{75}Se-LDL beta-apoprotein studies

		LDL-B Data						
Study	Lipoprotein Phenotype	Pool	Concentration	Turnover Rate	Turnover	FCR Day^{-1}	From IDL	Intravascular Distribution
		mg	*mg/100ml*	*mg/kg/day*	*mg/day*		%	%
Normolipidemic subjects								
1	N	1,495	43	7	571	0.38	70	66
2	N	834	38	12	641	0.76	61	66
3	N	1,073	48	10	567	0.53	56	66
4	N	1,184	44	10	683	0.57	95	63
5	N	1,013	34	7	532	0.52	76	67
Mean ± SD		1,119 ± 245	41 ± 5	9 ± 2	599 ± 61	0.55 ± 0.13	72 ± 15	66 ± 2
Hypertriglyceridemic patients								
6	IIb	1,929	59	16	1,248	0.65	100	34
7	IV	1,899	45	8	816	0.43	100	67
8	III	1,582	49	11	835	0.53	67	22
9	III	1,728	58	11	828	0.48	75	34
10	III	2,125	59	13	1,122	0.53	77	34
11	III	1,391	40	16	1,336	0.96	100	50
Mean ± SD		1,775 ± 264	52± 8	12 ± 3	1,031 ± 234	0.60 ± 0.19	87 ± 15	40 ± 16

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In our studies of hypertriglyceridemic patients, marked overproduction of apoB was characteristic, with major routes of direct synthesis of apoB entering via both the initial subclass and the terminal subclass of VLDL. These data are consistent with the results of Fisher et al. (3) in four hypertriglyceridemic subjects using [^{3}H]leucine as an endogenous tracer. The initial subclass of VLDL may represent the kinetic equivalent of the β-VLDL kinetically described by Berman et al. (7), and could correspond to the functional "hypertriglyceridemic" VLDL described by Gianturco et al. (2). The high affinity of such VLDL in binding in LDL receptors led these investigators to propose that an alteration in apoE isomorphic configuration and/or content may participate in the altered cellular interactions. Our demonstration of a selective increase in this VLDL kinetic subclass in hypertriglyceridemic patients is consistent with this hypothesis.

In addition, our studies indicate that the major degradative pathway of hypertriglyceridemic VLDL is direct catabolism, while the lipoprotein lipase-mediated delipidation conversion (29) to IDL represents a quantitatively less important catabolic pathway in our hypertriglyceridemic subjects. Nevertheless, lipoprotein lipase-mediated delipidation of VLDL to IDL is virtually threefold that seen in normal controls, leading to the augmented IDL-B turnover observed. Similar in vivo losses of VLDL beta-apoprotein prior to conversion to apoLDL have been previously reported with studies of autologous labeled VLDL (7, 30, 31) as well as with endogenously labeled VLDL (3, 13). This catabolic event is consistent with in vitro studies demonstrating that hypertriglyceridemic VLDL but not normal VLDL are bound, internalized, and degraded by normal fibroblasts (2) and mononuclear cells (6) primarily by the high affinity LDL receptor-mediated pathway. Only a quantitatively trivial amount of normal VLDL is bound and degraded by human cells in vitro, and this appears to be mediated by nonspecific and nonsaturable routes (2). While kinetic analysis of in vivo tracer data cannot prove that these events are occurring in vivo, they are quite compatible with the concepts described.

A linear relationship between production and concentration of beta-apoprotein within VLDL and IDL is demonstrated by our studies (Fig. 4). Sigurdsson, Nicoll, and Lewis (33) have previously observed a similar dependence of VLDL-B concentration upon beta-apoprotein production utilizing radioiodinated VLDL in man.

The physiological model confirmed by our studies is consistent with the concept that in the normal course of lipoprotein physiology, apoB-containing VLDL is secreted from the liver and converted to IDL and subsequently to LDL so that hepatogenous VLDL is the major source of circulating IDL and LDL. Malloy et al. (34) have reported that the normal VLDL-apoB is composed of three proteins of differing molecular weight, with a dominant species of molecular weight 549,000 (B-100). However, in the hypertriglyceridemic state, an abnormal subclass of "hypertriglyceridemic" VLDL may be secreted which is significantly degraded without prior conversion to IDL or LDL. In those patients with familial dysbetalipoproteinemia (Type III), this abnormal VLDL may be partially of intestinal origin, and contain an additional apoprotein B (B-48) of molecular weight and amino acid composition different from that of hepatic origin (35). In this situation, the abrupt degradation of VLDL prior to conversion to IDL/LDL, results in a dissociation between apoB-VLDL turnover and apoB-LDL turnover. ApoB-LDL concentration and turnover would be expected to be normal in this situation, as has been observed by other investigations in hypertriglyceridemic patients (4, 7, 13, 30, 36, 37) and confirmed by our data.

The methodology utilized in our studies and in those of Stahelin (26), Fisher et al. (3), and Phair et al. (13)

TABLE 5. Basal endogenous beta-lipoprotein turnover as evaluated by differing reported methodologies involving simultaneous considerations of both VLDL-B and LDL-B

Method	Number of Normal Subjects	VLDL-B Turnover	LDL-B Turnover
		mg/kg per day	
Compartmental analysis of ^{75}Se-labeled amino acid incorporation in vivo	5	18 ± 8[a]	9 ± 2
Two-pool model analysis of ^{125}I-LDL and ^{131}I-VLDL injection with urine/plasma evaluation of FCR (31)	8	26 ± 3	20 ± 2
First exponential analysis of FCR following injection of ^{131}I-VLDL and ^{125}I-LDL (14)	6	15 ± 4	12 ± 2
Exponential analysis of FCR after injection of ^{131}I-VLDL and ^{125}I-LDL (4, 32)	5	15 ± 3	7 ± 2
Two-pool model analysis of ^{131}I-VLDL injection and recovery within LDL (30)	3	9 ± 3	8 ± 2
Compartmental analysis of ^{125}I-VLDL injection and recovery within LDL (7)	4	8 ± 2	8 ± 2

[a] Mean ± SD.

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TABLE 6. Simultaneous evaluation of VLDL-B, IDL-B, and LDL-B in hypertriglyceridemia as evaluated by differing methodologies

Method	Number of Lipemic Subjects	VLDL-B Turnover	IDL-B Turnover	LDL-B Turnover	Clinical Type of Hyperlipemia
			mg/kg per day		
Endogenous tracer labeling					
Compartmental analysis of ^{75}Se-labeled amino acid incorporation in vivo	6	44–250	8–60	8–16	IIb, III, IV
Compartmental analysis of ^{3}H-leucine incorporation in vivo (3)	4	25–61	9–64	16–20	IIb, III, IV
Exponential analysis of ^{75}Se-labeled amino acid incorporation in vivo (26)	3	25–76			IIb, IV
In vitro tracer labeling					
Compartmental analysis of ^{125}I-VLDL injection and recovery within LDL (7)	9	5–17	5–28	5–29	III, IV
Two-pool model analysis of ^{131}I-VLDL and ^{125}I-IDL injection with recovery within LDL (30)	4	28–55	5–8[a]	9–19	IV, V
Exponential analysis of FCR after injection of ^{131}I-VLDL and ^{125}I-LDL (32)	5	17–52		8–19	Unclassified
	5	17–29		9–13	Familial combined hyperlipemia
Exponential analysis of FCR after injection of ^{125}I-VLDL (5)	6	15–21			Familial hypertriglyceridemia
	7	15–34			Familial combined hyperlipemia

[a] Only two of four patients studied.

involve endogenous lipoprotein labeling with an amino acid tracer. An alternative tracer methodology requires isolation of a lipoprotein, in vitro iodination, and re-injection (5, 7, 26, 30, 32). Both approaches involve assumptions concerning uniformity of label incorporation into precursor and product proteins, adequacy of mixing of labeled pools, alteration in metabolic behavior of "altered" lipoproteins produced by the isotope and/or in vitro handling, dilutional volumes of native versus labeled peptides, etc. In addition to these tracer considerations, there are limitations placed upon the data reduction by the method of kinetic analysis (38). Analysis by exponential representation of disappearance data permits quantitation of the metabolism of a single species with no assumptions relative to other interacting proteins of either a precursor or product nature. Analysis by model construction obligates an assumed interrelationship between precursors and products which may not be completely known in abnormal as well as normal states. Finally, the grouping of patients by clinical phenotype instead of genotype or pathophysiological identity places assumptions concerning similarity of lipoprotein abnormality which may be less defined than the complexities of tracer or analytical methodology.

In an attempt to compare these methods of approach, data obtained in normal subjects by these differing methodologies are tabulated in **Table 5.** It can be appreciated that no systematic difference between our amino acid tracer methodology and the in vitro tracer labeling methodology is apparent in terms of net apoB turnover (7, 14, 30–32). Similarly, data obtained by both endogenous labeling and in vitro labeling in hypertriglyceridemic subjects are tabulated in **Table 6** in which up to three of the major apoB lipoproteins have been examined. All investigators report a range of values for their lipemic populations, which are above either the normal range of their controls, the range for VLDL-B and IDL-B as observed in normal subjects by our endogenous labeling studies, and/or the range reported by the in vitro labeling methodology of Berman et al. (7) in normal subjects. The range of values for VLDL-B turnover by a given methodology, suggests wide differences between patients even when grouped by clinical phenotype. Without simultaneous investigations by both endogenous and in vitro tracer labeling, a systematic difference between these approaches is not obvious. The presence of a slowly turning-over B-VLDL which is catabolized without conversion to IDL and identified kinetically by Berman et al. (7) in their in vitro labeled VLDL studies was not found in our investigations of two Type III patients. Instead, direct catabolism of the initial lipid-laden VLDL-labeled pool (compartment-1) was markedly increased in these patients (Table 2). It would seem that B-VLDL and compartment-1-VLDL may be kinetically equivalent within the constraints and limitations of the two differing labeling methodologies used in our investigation (endogenous labeling) and that of Berman et al. (7) (in vitro labeling).

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APPENDIX

APPENDIX Kinetic parameters[a]

Pt.	λ0, 6	λ7, 6	λ6, 7	λ6, 5	λ0, 5	λ5, 4	λ0, 4
							hr⁻¹
Normolipidemic							
1	0.0160 ± 0.0019	0.0050 ± 0.0046	0.0100 ± 0.0049	0.20 ± 0.01	0.10 ± 0.01	0.200 ± 0.001	0.430 ± 0.001
2	0.032 ± 0.002	0.015 ± 0.003	0.030 ± 0.029	0.300 ± 0.004	0.100 ± 0.004	0.400 ± 0.006	0.230 ± 0.006
3	0.022 ± 0.001	0.0010 ± 0.0005	0.002 ± 0.002	0.30 ± 0.016	0	0.400 ± 0.020	.230
4	0.024 ± 0.003	0.0060 ± 0.0025	0.010 ± 0.053	0.150 ± 0.003	0.150 ± 0.003	0.630 ± 0.078	0
5	0.0220 ± 0.0001	0.0040 ± 0.0001	0.010 ± 0.004	0.300 ± 0.098	0.150 ± 0.005	0.26 ± 0.03	0.37 ± 0.03
Endogenous hypertriglyceridemic							
6	0.027 ± 0.001	0.0020 ± 0.005	0.001 ± 0.003	0.125 ± 0.009	0.225 ± 0.009	0.63 ± 0.04	0
7	0.018 ± 0.003	0.024 ± 0.030	0.050 ± 0.027	0.250 ± 0.005	0.050 ± 0.005	0.07 ± 0.01	0.56 ± 0.01
8	0.022 ± 0.001	0.090 ± 0.002	0.025 ± 0.086	0.016 ± 0.001	0.015 ± 0.001	0.100 ± 0.009	0.530 ± 0.009
9	0.020 ± 0.002	0.040 ± 0.002	0.0200 ± 0.0003	0.025 ± 0.002	0	0.10 ± 0.02	0.53 ± 0.02
10	0.022 ± 0.001	0.040 ± 0.001	0.020 ± 0.016	0.05 ± 0.01	0.25 ± 0.01	0.30 ± 0.01	0.33 ± 0.01
11	0.040 ± 0.002	0.001 ± 0.002	0.001 ± 0.009	0.08 ± 0.01	0.22 ± 0.01	0.15 ± 0.03	0.48 ± 0.03

[a] Values of the rate constants for the model which provide the optimal fit of the data are expressed ± change in the parameter which results in a 1% change in the residual of the fit of the data.
[b] Numerical designation of compartments: SG = ^{75}Se-labeled amino acid; 1–4 = VLDL cascade; 5 = IDL; 6 = LDL; 7 = extravascular LDL.
[c] λ3, 2 = λ4, 3 = λ2, 1.

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of apoB metabolism in man[b]

λ2, 1[c]	SG(1)	SG(4)	SG(5)	SG(6)	Delay λ6, 7	Delay SG(1)	Delay SG(4)
						min	
0.63	0	0.0500 ± 0.001	0	0.0010 ± 0.0001	120 ± 30	0	0
0.63	0	0.0180 ± 0.0001	0.0010 ± 0.0002	0.0060 ± 0.0001	70 ± 19	0	0
0.63	0.003 ± 0.0008	0.0070 ± 0.0001	0	0.120 ± 0.0001	100 ± 80	100 ± 41	0
0.63	0.0010 ± 0.0001	0.320 ± 0.0003	0.0070 ± 0.0003	0.0010 ± 0.0001	100 ± 15	0	1.0 ± 0.01
0.63	0.010 ± 0.218	0.0600 ± 0.0005	0	0.0060 ± 0.0006	100 ± 12	60 ± 89	0
0.63	0.020 ± 0.002	0.056 ± 0.002	0	0	0	30 ± 1	2.0 ± 0.03
0.63	0.05 ± 0.10	0.240 ± 0.003	0	0	100 ± 10	160 ± 140	3.0 ± 0.05
0.63	0.08 ± 0.01	0.140 ± 0.001	0.0020 ± 0.0001	0.0085 ± 0.0001	60 ± 43	70 ± 7	2.5 ± 0.02
0.63	0.050 ± 0.002	0.310 ± 0.004	0	0.0200 ± 0.0002	180 ± 55	25 ± 1	3.0 ± 0.2
0.63	0.030 ± 0.001	0.180 ± 0.001	0	0.0050 ± 0.0006	120 ± 35	20 ± 1	3.5 ± 1.1
0.63	0.04 ± 0.01	0.140 ± 0.008	0	0	0	55 ± 9	3.5 ± 0.3

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