Metabolic Studies in an Unusual Case of Asymptomatic Familial Hypobetalipoproteinemia with Hypoalphalipoproteinemia and Fasting Chylomicronemia

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ABSTRACT A new kindred with asymptomatic hypobetalipoproteinemia is reported. The proband, age 67, differs from previously described cases in several respects: (*a*) unusually low levels of low density lipoprotein (LDL) cholesterol (4–8 mg/dl); (*b*) normal triglyceride levels; (*c*) low levels of high density lipoprotein; (*d*) mild fat malabsorption; and (*e*) a defect in chylomicron clearance. On a high-carbohydrate diet his plasma triglyceride levels, instead of rising, actually fell. Turnover of triglycerides in very low density lipoproteins (VLDL) was low (2.8 mg/kg per h). Fractional catabolic rate of LDL protein was just above the normal range (0.655/d) but net turnover was <10% of normal (0.65 mg/kg per d). The half-life of his chylomicrons was 29 min, five times the normal value. Postheparin lipoprotein lipase activity was normal and apolipoprotein C-II, the activator protein for lipoprotein lipase, was present and functional. Apolipoprotein C-III$_1$, however, was not detected in the VLDL fraction, a finding previously reported in patients with abetalipoproteinemia. Fecal excretion of cholesterol was almost twice normal; total sterol balance was increased by ≅40%. The unusual features in the proband that distinguish him from previously described cases and from his affected first-degree relatives suggested that, in addition to the basic gene defect affecting LDL metabolism, he might have a second abnormality affecting clearance of chylomicrons and VLDL. The ratio of apolipoprotein E_3 to E_2 in his VLDL fraction was 0.93, just below the lower limit of normal, suggesting heterozygosity for E_3 deficiency. Whether or not this contributes to his hypertriglyceridemia remains to be established.

INTRODUCTION

Extremely low levels of plasma β-lipoproteins are encountered in at least two distinct inherited disease states (1). In "abetalipoproteinemia" no apoprotein B is detected in the plasma even by the most sensitive immunochemical assays. Ordinary methods fail to reveal low density lipoprotein (LDL),[1] very low density lipoprotein (VLDL), or chylomicrons. By concentrating the fraction with $d < 1.063$ it is possible to demonstrate lipoproteins but these are abnormal in composition and structure, i.e., they do not represent normal LDL or VLDL (1–4). This apparently total deficiency of lipoproteins containing apoprotein B is associated with a severe clinical syndrome, usually apparent in infancy (5). The syndrome is characterized by fat malabsorption, retinitis pigmentosa, neurologic involvement (including cerebellar ataxia and mental retardation), and acanthocytosis. The inheritance pattern is autosomal recessive, with parents having normal lipoprotein levels and none of the clinical manifestations.

In "hypobetalipoproteinemia" plasma LDL can be demonstrated but the levels are sharply reduced. This partial deficiency of LDL is not associated with the severe clinical manifestations characteristic of abetalipoproteinemia. Most patients are totally asymptomatic

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[1] *Abbreviations used in this paper*: apoA, B, C, and E, apolipoproteins A, B, C, and E; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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and the diagnosis is usually made by chance during a routine clinical work-up or in screening programs. Fat absorption is normal or only marginally impaired; acanthocytes are rarely seen; growth and development in infancy and childhood are almost always normal. A few patients have had neurological signs and symptoms but the other hypobetalipoproteinemic members of these kindreds were unaffected. The nature of the relationship between the neurologic changes and the hypobetalipoproteinemia remains unclear. This disorder is inherited in an autosomal dominant pattern.

Recently it has become clear that a double dose of the abnormal allele causing hypobetalipoproteinemia can produce a severe clinical picture not readily distinguishable from that of abetalipoproteinemia (6, 7). However, the clearly dominant inheritance of hypobetalipoproteinemia in these kindreds indicates that homozygous hypobetalipoproteinemia represents a mutation distinct from that in abetalipoproteinemia.

In this paper we present the results of intensive studies in an asymptomatic man in his seventh decade with a familial hypobetalipoproteinemic syndrome different from those previously reported in a number of respects: (*a*) extremely low LDL levels (4–8 mg LDL cholesterol/dl); (*b*) normal triglyceride levels; (*c*) low high density lipoprotein levels; (*d*) mild fat malabsorption; and (*e*) a chylomicron clearance defect.

METHODS

Patient. H.J.B., a retired Navy chaplain, was first seen in the La Jolla Lipid Research Clinic in September 1975 at age 64. He was referred because total plasma cholesterol, determined in the course of a routine screening examination, was 47 mg/dl. He has enjoyed remarkably good health. There was a history of labile hypertension during World War II but this never required treatment. The patient underwent thyroid surgery but has never been on replacement therapy and had a normal thyroxine level when referred. There was no history of childhood illness; no history of diarrhea or abnormal stools; no history of neurologic dysfunction, including muscle weakness, ataxia, paresthesias, anesthesia, or cranial nerve problems. Dietary history was unremarkable except that the patient prefers vegetables and avoids pastries, candies, and sweets. He does not smoke and is taking no medications.

When first seen in the fall of 1975 the proband had a fasting plasma that was slightly but definitely turbid. Lipoprotein quantification using standard Lipid Research Clinic methods (8) were as follows: total plasma cholesterol, 34 mg/dl; high density lipoprotein (HDL) cholesterol, 19 mg/dl; LDL cholesterol, 7 mg/dl; VLDL cholesterol, 8 mg/dl; total plasma triglycerides, 117 mg/dl. In December 1975 total plasma cholesterol was 34 mg/dl; HDL cholesterol, 23 mg/dl; LDL cholesterol, 3 mg/dl; VLDL cholesterol, 10 mg/dl. Fasting plasma triglycerides were 67 mg/dl but the plasma was again turbid even though the patient had been fasting for 17 h. Chylomicrons were demonstrated both by the presence of an opaque surface layer on plasma stored overnight in the refrigerator and by a lipid-staining band at the origin on agarose gel electrophoresis.

The patient was admitted to the Veteran's Administration Hospital, San Diego, on 6 May 1976. Physical examination revealed a well-nourished man 5 ft 6 in. tall and weighing 171 lb. Pulse, 72/min and regular; blood pressure, 160/90. There was no retinal pigmentation. Hearing was normal for his age. Heart and lungs were within normal limits. The liver was smooth and nontender, palpable 4 cm below the right costal margin, with a total liver span of 15 cm. The spleen was not felt. Rectal examination revealed an enlarged prostate consistent with benign prostatic hypertrophy. Neurological examination, performed by Professor W. C. Wiederholt, Chairperson of Neurosciences, was within normal limits. Specifically, there were no abnormal pyramidal, cerebellar, or posterior column signs and no peripheral neuropathy.

The following laboratory values were obtained: hemoglobin, 14.2 g/dl; hematocrit, 42.6%. Erythrocyte indices were normal and the morphology of most cells was normal except that a rare acanthocyte was noted. Leukocytes, 4,000/mm^3 with normal differential count. Fasting plasma glucose, 112 mg/dl; 2-h postprandial plasma glucose, 136 mg/dl. Normal values were found for blood urea nitrogen, uric acid, calcium, phosphorus, iron, electrolytes, creatinine, total bilirubin, alkaline phosphatase, lactic dehydrogenase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, creatine phosphokinase, bromosulfonphthalein retention, thyroxine, and free thyroxine index. Urinalysis was negative. Plasma protein electrophoresis was normal. Chest x ray was normal. Abdominal x ray showed multiple radiopaque shadows in the right upper quadrant consistent with cholelithiasis; abdominal echographic studies confirmed the presence of gallstones.

Electrocardiogram showed a normal sinus rhythm. There was left axis deviation and evidence of old anteroseptal myocardial infarction. Additional questioning elicited no history suggestive of infarction. A later exercise electrocardiogram (heart rate 160) elicited no symptoms and no ischemic signs.

Fecal fat in a 3-d stool collection was 13 g/d (dietary fat 100 g/d). Additional measurements on four 2-d stool collections, made in the course of measurements of sterol excretion, gave the following values for fat excretion: 15.3, 16.9, and 14.8 g/d.

Metabolic studies. The patient was studied in the Special Diagnostic and Treatment Unit of the Veteran's Administration Hospital and as an outpatient. He gave informed consent for all investigations.

The patient was hospitalized twice. During the first hospitalization of 3 wk he was fed a mixed solid food and liquid formula diet which has been described previously in detail (9). The diet provided 40% of calories as fat (in the form of lard), 45% of calories as carbohydrate, and 15% as protein; daily cholesterol intake was 93 mg/d. During the second hospitalization of 2 wk, the patient took an ad lib. solid food diet providing ≅40% of calories in the form of fat. After discharge, the patient was instructed to follow a low-fat diet for 3 wk. This diet consisted of low-fat vegetables, fruits, and skimmed milk; fat content was estimated to provide <10% of calories.

Plasma cholesterol and triglycerides were analyzed using a Technicon AutoAnalyzer (model II, Technicon Instruments Corp., Tarrytown, N. Y.) (8). Lipoprotein fractions were isolated by preparative ultracentrifugation using the methods of Havel et al. (10) unless otherwise specified. Electron microscopy of plasma lipoprotein fractions was carried out on negatively stained preparations prepared as described elsewhere (11).

Cholesterol balance studies were carried out according to methods previously described (12–14). Stools were collected for the last 10 d of the first hospitalization and 2-d pools were analyzed. Fecal neutral steroids and acidic steroids were determined by gas-liquid chromatography. To correct for losses of neutral steroids, β-sitosterol was given in small doses as an

internal standard and excretion of steroids was corrected for variations in fecal flow by use of chromic oxide as a marker. Administration of markers was begun at the time of hospitalization to ensure equilibration by the time stool collections were begun.

Qualitative tests for apolipoproteins were carried out by radial immunodiffusion (15) and by polyacrylamide gel electrophoresis (16). Quantitative measurements of apolipoprotein levels in whole plasma and in ultracentrifugal fractions were carried out by radial immunodiffusion and by double-antibody radioimmunoassay using methods previously described (17, 18). Isoelectric focusing for determinations of apolipoprotein (apo)E_3/apoE_2 ratios was done by the method of Warnick et al. (19). Total protein in fractions was determined by the method of Lowry et al. (20).

For measurement of LDL apoprotein turnover, LDL was prepared from the plasma of a normal donor negative for hepatitis B antigen and with a history of repeated uneventful plasma donations in the past. The LDL fraction (*d* 1.019–1.063) was iodinated with ^{125}I-Cl by a modification of the method of McFarlane (21) and Bilheimer et al. (22). The plasma and its fractions were handled with sterile precautions, passed through a 0.45-μm Millipore filter (Millipore Corp., Bedford, Mass.), and then tested for bacterial contamination and pyrogenicity before use. The recipient was started on KI, 900 mg/d, just before study and maintained on that dosage throughout. The labeled LDL (50 μCi) was injected after an overnight fast. Blood samples were drawn at frequent intervals during the first 24 h and daily thereafter. Previous studies have shown that little or no ^{125}I-LDL radioactivity is transferred to other lipoprotein fractions and analyses were based therefore on total radioactivity per milliliter of plasma. The plasma radioactivity decay curve was analyzed by the method of Matthews (23) for determination of fractional catabolic rate.

For 36 h before measurement of VLDL triglyceride turnover, fat was deleted from the patient's diet to eliminate possible ambiguities owing to chylomicronemia. Carbohydrate and protein intake were continued at the same level in frequent small feedings of liquid formula (24). For determination of the turnover of VLDL triglycerides, [^{14}C]glycerol was injected intravenously and blood samples were taken at intervals for isolation of the VLDL fraction. Lipids were extracted from each sample using isopropanol and phospholipids were adsorbed onto silicic acid. After saponification, the nonsaponifiable lipids were extracted into heptane. Free glycerol was determined in one aliquot of the aqueous phase and radioactivity was assayed by liquid scintillation counting of a second aliquot. Turnover was determined using multicompartmental analysis as described by Zech et al. (25).

Fibroblast cultures were initiated from a skin biopsy taken from the suprascapular region at the time of the first hospitalization. The cells were studied at the seventh passage. Methods for maintaining the cells in culture have been previously described (26). LDL isolated from pooled normal human plasma was iodinated as described above and its surface binding, internalization, and degradation were determined in normal cultured fibroblasts and in the patient's fibroblasts. In addition, competition between LDL isolated from the patient and iodinated normal LDL was studied and compared with competition between unlabeled LDL from a normal donor and iodinated LDL prepared from pooled normal human plasma.

RESULTS

Plasma lipids and lipoproteins. Total plasma cholesterol and triglyceride values during three different dietary periods in the course of metabolic studies are shown in Table I. Total plasma cholesterol remained very low on all diets, possibly somewhat lower on the low-fat diet. The range was from 28 to 48 mg/dl. Total plasma triglycerides were more variable. The mean values on diets containing 40% of calories as fat were within the normal range. Interestingly, the low-fat diet, which provided ≅80% of calories in the form of carbohydrate, was not associated with a rise in plasma triglycerides; in fact, the mean triglyceride value on this high-carbohydrate diet was significantly lower than on the diets providing 40% of calories as fat.

While on the low-fat diet, the patient was given an oral meal of 100 g of safflower oil. The cholesterol and triglyceride content of whole plasma and of ultracentrifugally isolated lipoprotein fractions in the fasting state and at intervals after the fat meal are shown in Table II. Total plasma triglyceride rose considerably,

TABLE I
Effects of Diet on Plasma Cholesterol and Triglycerides in Patient H.J.B.

Diet	Total plasma cholesterol	Total plasma triglycerides
	mg/dl	*mg/dl*
Formula supplemented with solid foods (40% calories from fat)	40±1.5 (6)*	91±14 (6)
Ad lib. solid foods (≅40% of calories from fat)	42±2.8 (4)‡	103±16 (4)
Low-fat diet (≅5% of calories from fat, 80% from carbohydrates)	33±1.9 (5)§	61±11 (5)

* Six determinations over the last 21 d of a 26-d period. Mean ±SEM.
‡ Four determinations over the last 9 d of a 14-d period. Mean ±SEM.
§ Five determinations over the last 15 d of a 21-d period. Mean ±SEM.

TABLE II
Plasma Lipids in Lipoprotein Fractions after a 100-g Oral Fat Load

	Whole plasma		Chylomicrons		VLDL		IDL/LDL*		HDL	
Time	C‡	TG	C	TG	C	TG	C	TG	C	TG
h	*mg/dl*									
Zero	34	38	0	2	7	24	8	5	16	4
4	36	68	0	29	4	13	3	11	23	15
5	31	137	2	86	4	21	3	13	22	17
6	33	234	4	191	3	19	2	10	23	14

* IDL/LDL = fraction of *d* 1.006 – 1.063.
‡ C, cholesterol; TG, triglycerides.

from 38 mg/dl initially to a peak of 234 mg/dl at 6 h. Most of this elevation was accounted for by the triglyceride content of the chylomicron fraction. Total plasma cholesterol did not change significantly. Triglyceride content of the VLDL fraction did not increase. However, there were small but consistent increases in the triglyceride content of the intermediate density lipoprotein (IDL)/LDL fraction (*d* 1.006–1.063) and of the HDL fraction.

Apolipoproteins. On two occasions the total apoB content of whole plasma determined by radioimmunoassay, was found to be 5.8 and 6.6 mg/dl, respectively. The apoB content of the lipoprotein subfractions is shown in Table III. The apoB content in the *d*-1.006–1.063 fraction was 2.4 mg/dl, <3% of the normal value. The total protein content of this fraction was 3 mg/dl, in reasonably good agreement with the apoB content determined by radioimmunoassay.

The VLDL fraction contained only 0.9 mg/dl of apoB despite the relatively normal total triglyceride concentration in the VLDL fraction, i.e., the apoB content was abnormally low. The total protein in this fraction was 2.8 mg/dl, suggesting that apoB accounted for approximately one-third of the total protein, in good agreement with published values for normal VLDL (27). As shown in Fig. 1 the VLDL fraction was relatively enriched in apoE ("arginine-rich protein"). The band running just behind apoE has not been further characterized. Isoelectric focusing on two occasions gave $apoE_3/apoE_2$ ratios of 0.96 to 0.90. Ratios below 1.0 are considered to be in the intermediate range found in subjects heterozygous for $apoE_3$ deficiency (19). It is also evident from Fig. 1 that there is a marked deficiency of $apoC\text{-}III_1$ accompanied by a relative increase in $apoC\text{-}III_2$.

TABLE III
Apoprotein Concentrations in Whole Plasma and in Lipoprotein Fractions

Apoprotein and plasma fraction	Concentration in H.J.B.	Normal subjects*
Apo B		
Whole plasma	5.8	85±18‡
Chylomicrons (fasting)	0.04	—
VLDL	0.9	8±7
IDL/LDL	2.4	76±16
HDL	2.2	2.0
$d > 1.25$	0.3	—
Apo A-I		
Whole plasma	77	120±20
Apo A-II		
Whole plasma	17	33±5

* Mean values for normolipidemic subjects determined in surveys by the Northwest Lipid Research Clinic (17, 18).
‡ SD.

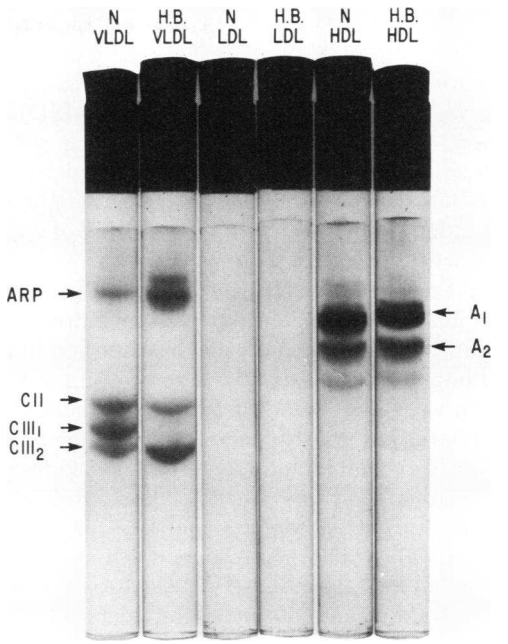

FIGURE 1 Urea-polyacrylamide gel electrophoresis of the apoproteins in VLDL, LDL, and HDL fractions from patient H.J.B.'s fasting plasma.

The HDL fraction (*d* 1.063–1.21) contained 2.2 mg/dl of apoB, a value not different from that found in the same fraction isolated from normal individuals (2.0 mg/dl) and possibly reflecting the presence of Lp (a) (17). The concentrations of apoA-I and apoA-II were both lower than the normal values (77 vs. 120 mg/dl and 17 vs. 33 mg/dl, respectively (18). As shown in Fig. 1, the pattern of apoproteins in HDL was normal.

Properties and turnover of VLDL. On electron microscopy the VLDL fraction was not unusual except for the presence of some particles with diameters larger than those usually found in this fraction. The mean diameter was 375±127 Å (SD). Kane et al. (28) report a mean VLDL diameter of 364 Å in 12 normal subjects but 98% of the molecules had diameters between 250 and 500 Å; in the present case 9% had diameters >500 Å, i.e., there was considerable skewing to the right. The larger particles may represent residual chylomicrons. Paper and agarose gel electrophoresis showed a predominant pre-β-band with some trailing to the origin.

Turnover of the triglycerides in VLDL was determined by injecting [^{3}H]glycerol and following the specific radioactivity of the glycerol moiety in VLDL triglycerides as a function of time (24, 25). The peak of the specific radioactivity curve was delayed and only ≅25% of the value reached in normolipidemic subjects. The rate of decay was also much slower. From multicompartmental kinetic analysis generously carried out for us by Dr. Loren Zech and Dr. Mones Berman (National Institutes of Health), the production rate of VLDL tri-

glycerides was estimated to be 2.8 mg/kg per h, far below the value of 11.3±3.9 (SD) mg/kg per h found in 18 normal subjects (25). Fractional catabolic rate, 0.11 h, was also low (normal, 0.162±0.008 [SD]).

Properties and turnover of LDL. The *d*-1.019–1.063 fraction was prepared from 84 ml of plasma. Gel electrophoresis showed a single band with beta mobility and radial immunodiffusion gave a line of apparent identity with normal LDL.

This preparation of LDL was functionally compared with normal LDL by examining its interaction with the high affinity LDL receptor site on the membrane of normal human fibroblasts (29). The binding, internalization, and degradation of ^{125}I-labeled normal LDL was determined as previously described (26) in the presence or in the absence of added unlabeled LDL either from a normal subject or from H.J.B. As shown in Fig. 2, the degradation of the labeled normal LDL was reduced ≅70% by unlabeled normal LDL and to the same extent by unlabeled LDL from H.J.B. The same was true with respect to binding and internalization (data not shown).

We considered the possibility that H.J.B. might be degrading LDL at an abnormally high rate and studied his own skin fibroblasts in culture from this point of view. Degradation of LDL (10 μg/ml) by the patient's cells grown in 10% fetal calf serum and incubated for 24 h in lipoprotein-deficient serum for 24 h was slightly less than that seen in a normal fibroblast line. We also tested whether the patient's cells would show the normal suppression of LDL receptor number in response to LDL in the medium (29). After a 24-h exposure to LDL (50 μg/ml), degradation rate decreased by 79% in H.J.B. cells and by 76% in control fibroblasts (Fig. 3).

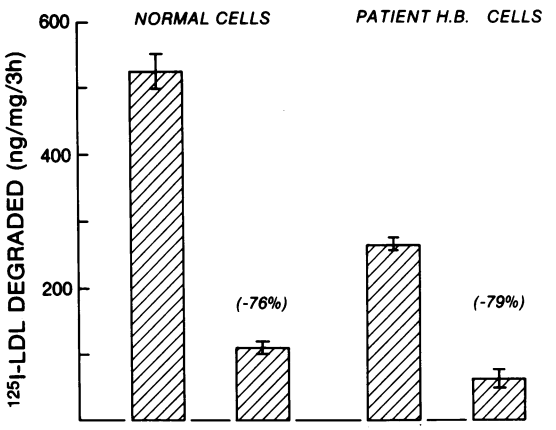

FIGURE 3 Comparison of normal skin fibroblasts and fibroblasts from H.J.B. with respect to suppression of LDL degradation resulting from a 24-h incubation with normal LDL. The cells were incubated with normal ^{125}I-LDL (10 μg/ml) for 3 h, either after a 24-h incubation in lipoprotein-deficient medium or after an additional 24-h incubation with normal LDL (50 μg/ml). The latter was removed by washing the monolayer before addition of ^{125}I-LDL and degradation was measured as previously described.

Turnover of LDL was determined using ^{125}I-LDL prepared from the plasma of a normal donor. The time-course of disappearance of ^{125}I from the plasma is shown in Fig. 4, the smooth curve representing the best-fit theoretical curve assuming the usual biexponential decay. Fractional catabolic rate was 0.582/d. When three exponential functions were allowed, calculated fractional catabolic rate was 0.655/d, at the very upper limit of values reported in normal subjects (0.26–0.63/d) (30–32). However, because of the extremely low pool

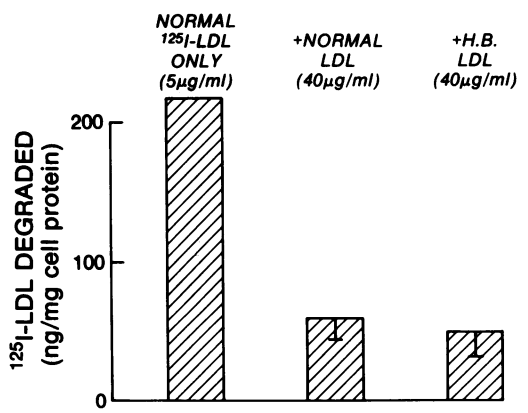

FIGURE 2 Inhibition of the degradation of normal ^{125}I-LDL (5 μg protein/ml) by unlabeled normal LDL or unlabeled H.J.B. LDL (40 μg protein/ml). Normal human skin fibroblasts were incubated overnight in medium containing 10% lipoprotein-deficient calf serum. The medium was changed and normal ^{125}I-LDL was added alone, with normal unlabeled LDL or H.J.B. LDL. After 3 h, TCA-soluble ^{125}I in the medium was measured as described elsewhere (26).

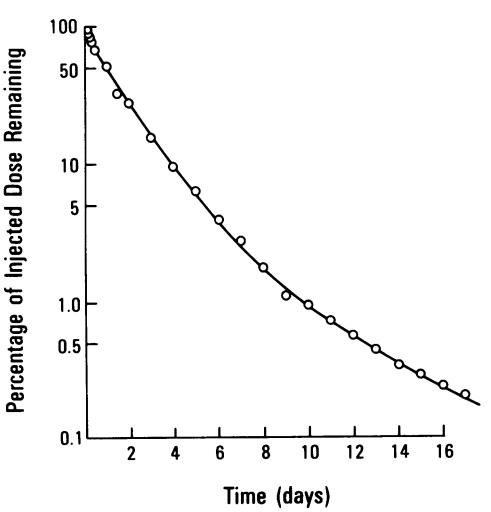

FIGURE 4 Plasma ^{125}I disappearance curve after injection of normal ^{125}I-LDL. The smooth curve represents the best-fit computer-generated curve using biexponential analysis. The experimental data points are shown by the circles.

size, the total calculated daily production of LDL apoB was only 0.65 mg/kg per d (normal, 8–20 mg/kg per d).

Properties and turnover of chylomicrons. Because of the patient's fasting chylomicronemia it was of interest to assess the severity of his clearance defect. This was done by the method of Grundy and Mok (33) in which triglyceride is continuously administered intraduodenally over a 6–8-h period and plasma samples are taken at intervals for determination of chylomicron triglyceride levels. After 5 h, plasma triglyceride levels reach a steady-state value. At this time the rate of chylomicron clearance from plasma equals the rate of chylomicron input from the intestine. Knowing the rate of intraduodenal fat infusion (and assuming complete absorption) and the estimated total plasma volume, one can calculate the fractional clearance and half-life of chylomicron triglycerides. The data in patient H.J.B. (not shown) yielded a half-life for chylomicron triglycerides of 29 min, more than five times the value in normoglyceridemic controls (5.4±2.9 min) (33).

The apparent chylomicron clearance defect and the low fractional catabolic rate for VLDL triglycerides suggested the possibility of a lipoprotein lipase deficiency. However, both postheparin lipoprotein lipase and hepatic triglyceride lipase, although below the mean normal levels, were found to be within normal limits (10 and 15 μeq FFA/ml per h, respectively, vs. normal ranges of 8–50 and 3–100) (34). ApoC-II was present (Fig. 1) and the patient's plasma was able to activate lipoprotein lipase in postheparin plasma of a normal subject.

Sterol balance. If endogenous cholesterol biosynthesis by peripheral tissues is normally suppressed by LDL, it should be abnormally high in H.J.B. As shown in Table IV, neutral steroid excretion on a low intake of dietary cholesterol was almost twice normal. Acidic steroid excretion, however, was somewhat below normal so that net cholesterol balance was increased ≅40%.

Family study. The proband's mother, age 92, is well except for mild diabetes. She had 12 siblings, 2 of whom are still living and in good health at 82 and 96 yr, respectively. The other siblings died of cancer or unknown cause; none died of heart disease. The patient's father died at age 86.

Lipoprotein data on the H.J.B. kindred so far available are summarized in Table V. The proband's mother has hypobetalipoproteinemia (LDL cholesterol 46 mg/dl) but much less striking than that of H.J.B. Lipoprotein quantification has been carried out on all living siblings (one sister died in her 20s of rheumatic heart disease) and both children of the proband. One brother (A.L.B.) and one sister (J.Y.G.) have hypobetalipoproteinemia as striking as that in H.J.B.—LDL cholesterols of 4 and 5 mg/dl, respectively. Both differ, however, in having low plasma triglyceride levels and normal HDL levels. Neither showed fasting chylomicronemia. Three additional siblings (J.L.P., R.W.B., and B.R.V.) have LDL cholesterol levels below 100 mg/dl and total plasma triglycerides below 40. The remaining three siblings have LDL cholesterol levels in the low normal range and normal total plasma triglyceride levels.

Both daughters have hypobetalipoproteinemia (LDL cholesterol levels 29 and 40 mg/dl). They also have low triglyceride levels (31 and 21 mg/dl) and normal to high HDL cholesterol levels (67 and 51 mg/dl). Thus the proband differs from all of the other affected members of the kindred in having chylomicronemia, normal plasma triglyceride levels, and low HDL levels.

None of the members of the kindred gave histories of abnormal childhood development, steatorrhea, visual disturbances, or neurologic symptoms.

TABLE IV
Sterol Balance Data

	Control subjects*	Patient H.J.B.‡
	mg/kg/d	
Cholesterol intake	1.8	1.24
Fecal neutral steroids	6.5±1.8§	12.2±1.9
Fecal acidic steroids	4.9±1.8	2.5±0.52
Total fecal steroids	11.4±2.5	14.7±2.3
Sterol balance	9.6±2.3	13.5±2.3

* n = 14.
‡ Mean of five 2-d collections.
§ SD.

TABLE V
Plasma Lipid Levels in the H.J.B. Kindred

	Sex	Age	Cholesterol			Total plasma triglycerides
			Total plasma	LDL	HDL	
			mg/dl			
Mother						
H.B.B.	F	89	102	46	46	60
Proband						
H.J.B.	M	66	40	3–8	23	91
Siblings						
A.L.B.	M	47	59	5	50	29
J.Y.G.	F	61	49	4	37	24
J.L.P.	F	64	110	62	46	26
R.W.B.	M	57	129	73	50	23
B.R.V.	F	59	154	83	71	34
E.E.B.	M	54	156	103	38	71
C.B.	M	68	177	120	46	118
E.E.V.	F	51	204	149	47	113
Children						
C.V.B.	F	34	102	29	67	31
S.G.S.	F	29	92	40	51	21

DISCUSSION

Patient H.J.B. differs from most previously described cases of familial hypobetalipoproteinemia in several respects. First, his LDL cholesterol levels (4–8 mg/dl) are below those generally reported, as are the levels in two of his affected siblings (4 and 5 mg/dl, respectively). Second, despite the extremely low LDL levels, his triglyceride levels are in the normal range (91 mg/dl on an ad lib. diet). One patient in the kindred described by Richet et al. (35) and four patients in the kindred described by Tamir et al. (36) had LDL cholesterol levels below 10 mg/dl but these patients all had low triglyceride values. In most previously reported cases the degree of hypobetalipoproteinemia is paralleled by the degree of hypotriglyceridemia. Third, HDL cholesterol levels in H.J.B. are distinctly low—about one-half normal. Fourth, although there is no history of symptomatic malabsorption, fecal fat levels are slightly but definitely increased in H.J.B. (13–17 g/d). Finally, he shows fasting chylomicronemia and a documented defect in chylomicron clearance. Fredrickson et al. (37) noted fasting chylomicronemia in several members of one kindred when high-fat meals were fed the preceding day and considered the possibility that a delay in fat absorption might account for it. In the present case, both impaired chylomicron clearance and delayed fat absorption may contribute.

The hypobetalipoproteinemia in H.J.B. appears to result from a marked reduction in the rate of LDL production. Fractional catabolic rate was at the upper limit of the normal range but not high enough to account for the low steady-state LDL levels. Daily production of LDL apoprotein (0.65 mg/kg per d) was <10% of normal. These data were obtained using homologous labeled LDL prepared from plasma of a normal donor and may not reflect faithfully the kinetics of H.J.B.'s endogenous LDL. However, attempts to demonstrate abnormalities in his LDL have thus far been negative. His LDL gave a line of identity with normal LDL in immunodiffusion and it appeared to bind normally to LDL receptor sites on normal human skin fibroblasts. Furthermore, studies of skin fibroblasts from patient H.J.B. showed that his own LDL receptors are within normal limits and under normal regulation by the LDL levels to which they are exposed. Thus, it seems reasonable to accept the low value for LDL production derived from the use of homologous LDL. Levy et al. (38) and Sigurdsson et al. (39) have reported low LDL apoB synthetic rates in two other familial hypobetalipoproteinemia kindreds using isologous LDL. Both absolute and fractional catabolic rate for VLDL triglycerides were low. VLDL apoB turnover has not been directly determined but if we make the approximation that fractional catabolic rate for VLDL apoB is the same as that for VLDL triglyceride (it will actually be somewhat lower) we can estimate apoB turnover to be about 0.97 mg/kg per d. This low value results from both a low fractional catabolic rate and a low apoB:triglyceride ratio. In normal subjects apoB turnover in VLDL is nearly the same as that in LDL i.e., 10–20 ml/kg per d (22, 40, 41). The calculated value in H.J.B. is distinctly low, only slightly greater than his apoB transport in LDL (0.65 mg/kg per d). Thus the results are compatible with the interpretation that VLDL production is abnormally low and that most of the patient's plasma LDL arises from VLDL. Sigurdsson et al. (39) measured both VLDL and LDL apoB turnover in a single patient with hypobetalipoproteinemia. The fractional catabolic rate for LDL was within normal limits whereas that for VLDL was a little >50% of normal; absolute synthetic rates of apoB were 8.7 mg/kg per d in VLDL and 5.96 mg/kg per d in LDL, values almost 10 times those in the present case.

On a fat-free, high-carbohydrate diet H.J.B. failed to show carbohydrate-induced elevation of plasma triglycerides. Fredrickson et al. found normal carbohydrate induction in the affected members of their kindred (kindred 4 [37]). An absence of carbohydrate induction might be expected if our patient's capacity to secrete apoB is already fully expressed. However, he actually showed a decrease in plasma triglyceride levels on the fat-free diet, suggesting the possibility that a significant part of his VLDL may have its origin from chylomicrons.

The ability of LDL to regulate cholesterol biosynthesis (at the hydroxymethylglutaryl-CoA reductase step) in cultured cells of animal and human origin is well established (42). There is evidence from animal studies indicating that this suppression is operative in vivo (43, 44) but it is not known with certainty whether or not synthesis in peripheral tissues in man is normally suppressed by LDL. Total sterol output in patients with abetalipoproteinemia is increased (45) but much or all of the increase might be due to increased hepatic synthesis due to the accompanying malabsorption and failure to make chylomicrons. The patient described here presented the unusual combination of normal (or nearly normal) fat absorption and chylomicron formation but almost total absence of LDL. If the relationship between intravascular and extravascular LDL concentrations in H.J.B. is similar to that in normal and hyperlipidemic subjects (46), his extravascular LDL cholesterol levels would be ≅0.5 mg/dl or 5 μg/dl, i.e., below the level needed to saturate the high affinity receptors (42). The finding of a 40% increase in total sterol output is compatible with the conclusion that cholesterol synthesis is normally suppressed by LDL. Because of the wide range of normal values, however, a firm conclusion will require additional studies of patients of this kind.

Hypobetalipoproteinemia (LDL cholesterol concentration >2 SD below the normal) was present in six

siblings (including the proband) of the nine examined: three males and three females were affected. The proband's mother and both of his children were also affected. The findings are compatible with autosomal dominant inheritance. Three of the affected siblings had LDL cholesterol levels below 10 mg/dl whereas the other three had levels ranging from 62 to 83 mg/dl. In the absence of other information one would have to seriously consider the possibility that H.J.B. and his siblings, J.Y.G., and A.L.B., are actually homozygotes (i.e., have a double dose of a dominant allelic mutation) whereas his mother, the other three siblings and his daughters are heterozygotes. However, in previously described kindreds the homozygotes have been characterized by an absence of detectable LDL and by the signs and symptoms of abetalipoproteinemia in early childhood (5–7). Only if the mutation in the H.J.B. kindred is distinct from that in the previously described kindreds does the suggestion of homozygosity have weight.

H.J.B. differs both from the other affected members of his kindred and from most previously described hypobetalipoproteinemic patients in that his triglyceride levels are normal and he displays fasting chylomicronemia on many occasions. In view of his demonstrated defect in chylomicron clearance and in the absence of any obvious environmental factors, we considered the possibility of an abnormality in apoC-II (47) but found none. The low $apoE_3/apoE_2$ ratio in his VLDL may be relevant to his clearing defect. Utermann and co-workers have shown that patients with familial dysbetalipoproteinemia, associated with accumulation of chylomicron "remnants", have complete or nearly complete $apoE_3$ deficiency (48). Interestingly, some subjects with the complete deficiency of $apoE_3$ do not have hyperlipoproteinemia although their plasma shows the characteristic abnormal "floating" betalipoprotein (49). These patients have abnormally low plasma LDL levels. Intermediate degrees of $apoE_3$ deficiency (heterozygous state) are extremely common (49, 50) but are not necessarily associated with hyperlipoproteinemia. It is possible that the low $apoE_3/apoE_2$ ratio in our patients accounts for some of the unusual features in his case. A deficiency of apoC-III_1 has been reported in abetalipoproteinemia (4, 51) but this is the first reported example in apoC-III_1 deficiency in hypobetalipoproteinemia. Whether it represents a primary defect or a secondary consequence of low LDL levels is not known.

The nature of the mutant allele in hypobetalipoproteinemia remains unknown. The present studies and those in two other kindreds (38, 39) show that it leads to a marked reduction in the rate of apoB production but how this is effected is not known. Malloy and Kane recently described a child with hypobetalipoproteinemia and normal triglyceride levels (52). The clinical syndrome in this case differs in a number of respects from that of the present case and the apoB appears to be structurally abnormal. Structural abnormalities in the LDL have not been demonstrated in our case or other cases but further efforts are in order to look for subtle abnormalities. It is equally possible that any one of the many steps in VLDL biosynthesis, packaging or secretion may be involved in a regulatory sense. This might help account for the observed variability in expression.

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