Variable expression of familial heterozygous hypobetalipoproteinemia: transient malabsorption during infancy

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Abstract Rare instances of symptomatic fat malabsorption have been reported in patients with heterozygous hypobetalipoproteinemia, but with an unclear pathogenesis. An 8-month-old boy with chronic diarrhea and failure to thrive was found to have abnormally low plasma total cholesterol (85 mg/dl), LDL-cholesterol (48 mg/dl), apoB (52 mg/dl), apoA-I (53 mg/dl), and vitamin E (0.22 mg/dl). Decreased plasma LDL-C and apoB were noted in the father (34 and 40 mg/dl, respectively), as well as several other family members. Fasting triglycerides were normal but did not increase normally in response to a fat meal test. Lipoprotein composition showed an abnormal profile of very low density (VLDL, d 1.006 g/ml), low density (LDL, d 1.063 g/ml), and high density (HDL, d 1.21 g/ml) lipoproteins. A fasting jejunal biopsy revealed lipid-laden enterocytes. Electron microscopy of the jejunal biopsy revealed the absence of lipid particles in the intercellular spaces after a fat meal. Jejunal explants cultured with [^{14}C]palmitate and [^{3}H]leucine showed limited synthesis of triglycerides and apolipoproteins (36 and 42% of controls, respectively), whereas the father's results were close to normal. At 1 year of age, improvement in intestinal fat absorption was accompanied by the presence of chylomicrons in the intercellular space, concomitant with the enhanced synthesis of lipids and apoB by jejunal explants. These data provide evidence that heterozygous hypobetalipoproteinemia may present early in life as transient, symptomatic lipid malabsorption. The mechanisms responsible for improved lipid transport despite persistent hypobetalipoproteinemia remain to be established.—**Levy, E., C. C. Roy, L. Thibault, A. Bonin, P. Brochu, and E. G. Seidman.** Variable expression of familial heterozygous hypobetalipoproteinemia: transient malabsorption during infancy. *J. Lipid Res.* 1994. **35:** 2170–2177.

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Familial hypobetalipoproteinemia is a rare congenital, dominant transmitted disorder of lipoprotein metabolism (1) characterized by low levels of apolipoprotein B (apoB) -carrying lipoproteins (VLDL, IDL, and LDL). The homozygous form of hypobetalipoproteinemia causes severe hypocholesterolemia, resembling other rare genetic lipid transport diseases such as abetalipoproteinemia (2, 3) and chylomicron retention disease (4, 5). These disorders are characterized by fat-filled enterocytes, intestinal malabsorption, acanthocytosis, absent or decreased apoB-containing lipoproteins, and severe vitamin E deficiency (1).

Heterozygous hypobetalipoproteinemia is a less severe form. It is usually asymptomatic and most often detected during family screening or population studies (1). However, occasional patients with heterozygous familial hypobetalipoproteinemia present with symptoms and signs of fat malabsorption and secondary neurological abnormalities (6). Occasionally, affected family members differ from the proband with respect to steatorrhea, plasma lipids and lipoproteins, vitamin E status, and clinical manifestations, despite similar apoB levels (6–8). Currently available information suggests a heterogeneity of etiological defects (6). More than 20 different mutations have been reported, which were associated with defective synthesis of apoB-containing lipoproteins by either the liver or intestine (9). The variant forms of hypobetalipoproteinemia have been described by nonsense mutations and nucleotide and exon deletions (9).

In this report, we investigated a child with heterozygous hypobetalipoproteinemia in whom the clinical manifestations and biochemical abnormalities improved with time. The purpose of this study was to examine the relationship between apoB and gastrointestinal manifestations of heterozygous familial hypobetalipoproteinemia.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; PL, phospholipids; FC, free cholesterol; CE, cholesteryl ester; PR, protein.

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CASE REPORT

An 8-month-old French Canadian male was hospitalized for evaluation of malabsorption. He was born at 35 weeks by repeat C-section to nonconsanguinous parents. There was a history of chronic diarrhea since the first week of life, characterized by 4–5 foul-smelling, greasy stools per day. The child was breast-fed until 4 months of age. Subsequently, multiple formula changes failed to improve the diarrhea. Past medical and family histories were noncontributory. Physical examination on admission was remarkable only for signs of acute malnutrition (weight <3%, height 25%). Complete neurological examination was normal, as were the EMG and peripheral nerve conduction studies. However, retinal electrophysiological studies showed an abnormally delayed evoked potential response (160–200% of normal).

Laboratory investigations revealed low serum carotene (0.24, N 1.3-6.3 μmol/l), vitamin A (0.75, N 1.05–2.8 μmol/l), and vitamin E (4.9, N 11.6-28 μmol/l). The vitamin D level was normal, but the prothrombin time was slightly prolonged (15/11 sec, INR 1.36). Liver transaminases were slightly elevated (AST 119, N 10–80 U/l; ALT 142, N 5–25 U/l), while bilirubin and alkaline phosphatase were both normal. Steatorrhea was confirmed by a 72-h stool collection (16.6% of ingested fat malabsorbed). A sweat chloride was normal, as were pancreatic enzymes in duodenal fluid (chymotrypsin, lipase). Plasma lipid profile revealed hypocholesterolemia, and his plasma lipoprotein pattern was suggestive of hypobetalipoproteinemia (**Table 1**).

Fasting jejunal biopsy (**Fig. 1A**) revealed pale, vacuolated enterocytes in the upper two thirds of the villi, without villus atrophy. Oil red-O stain confirmed marked lipid vacuolization of the villus epithelium. Electron microscopy of a repeat jejunal biopsy 90 min after a fat meal depicts the marked lipid vacuolization of the enterocytes (Fig. 1B), and the notable absence of chylomicrons in the intracellular spaces (Fig. 1C).

MATERIALS AND METHODS

After a 12-h fast, blood was collected from the patient and family members into tubes containing Na_2 EDTA as anticoagulant. Plasma was isolated by centrifugation at 3000 rpm for 30 min at 4°C. The lipoprotein fractions were isolated by sequential ultracentrifugation (10) with a Beckman model L5-65 ultracentrifuge as previously reported (2, 5). Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were separated at densities 1.006 and 1.063 g/ml, respectively, by centrifugation at 40,000 rpm with a 50 Ti rotor for 18 h at 5°C. The high density lipoprotein (HDL) fraction was obtained by adjusting the LDL infranatant to 1.21 g/ml followed by a 48-h centrifugation at the same speed. Concentrations of cholesterol, phospholipid, triglyceride, protein, apoA-I, and apoB were determined by previously described methods (4, 11). A standard fat meal test was performed after the ingestion of 50 g of fat per 1.73 m^2 in the form of flavored cream, and plasma triglycerides were determined at defined periods (5).

Explant cultures of jejunal biopsy specimens were set up by methods previously described (2, 5). Briefly, the intestinal specimens were placed in petri dishes containing RPMI-1640 medium (Gibco, Grand Island, NY) supplemented by lipoprotein-deficient serum. [^{14}C]palmitic acid and [^{3}H]leucine were added to study lipid and apoB synthesis, respectively. In experiments with [^{14}C]palmitate, after the 18-h incubation, the biopsy homogenates were

TABLE 1. Plasma lipids, lipoproteins, and apolipoproteins in familial hypobetalipoproteinemia

Subjects	Triglycerides	Cholesterol	LDL-C	HDL-C	ApoA-I	ApoB
			mg/dl			
1. Proband						
8 mo	76	87	48	16	53	52
12 mo	137	119	74	23	67	62
2. Brother	141	196	121	69	164	113
3. Mother	324	233	156	37	127	164
4. Father	177	114	34	48	81	40
5. Paternal uncle	19	62	23	25	60	13
6. Paternal uncle	43	89	36	40	75	19
7. Paternal uncle	51	99	43	40	113	35
8. Paternal uncle	152	133	65	46	151	40
9. Paternal uncle	107	138	96	25	101	51
10. Paternal uncle	59	102	13	52	115	12
11. Paternal uncle	192	203	135	32	106	71
12. Paternal aunt	142	226	124	41		
13. Paternal aunt	27	170	68	81	173	37
14. Paternal aunt	28	146	69	70	162	32
15. Paternal aunt	71	188	103	70	182	60
Pediatric controls	48-115	130-180	90-130	48-70	95-130	95-125
Adult controls	64-110	180-226	92-125	39-72	105-167	95-131

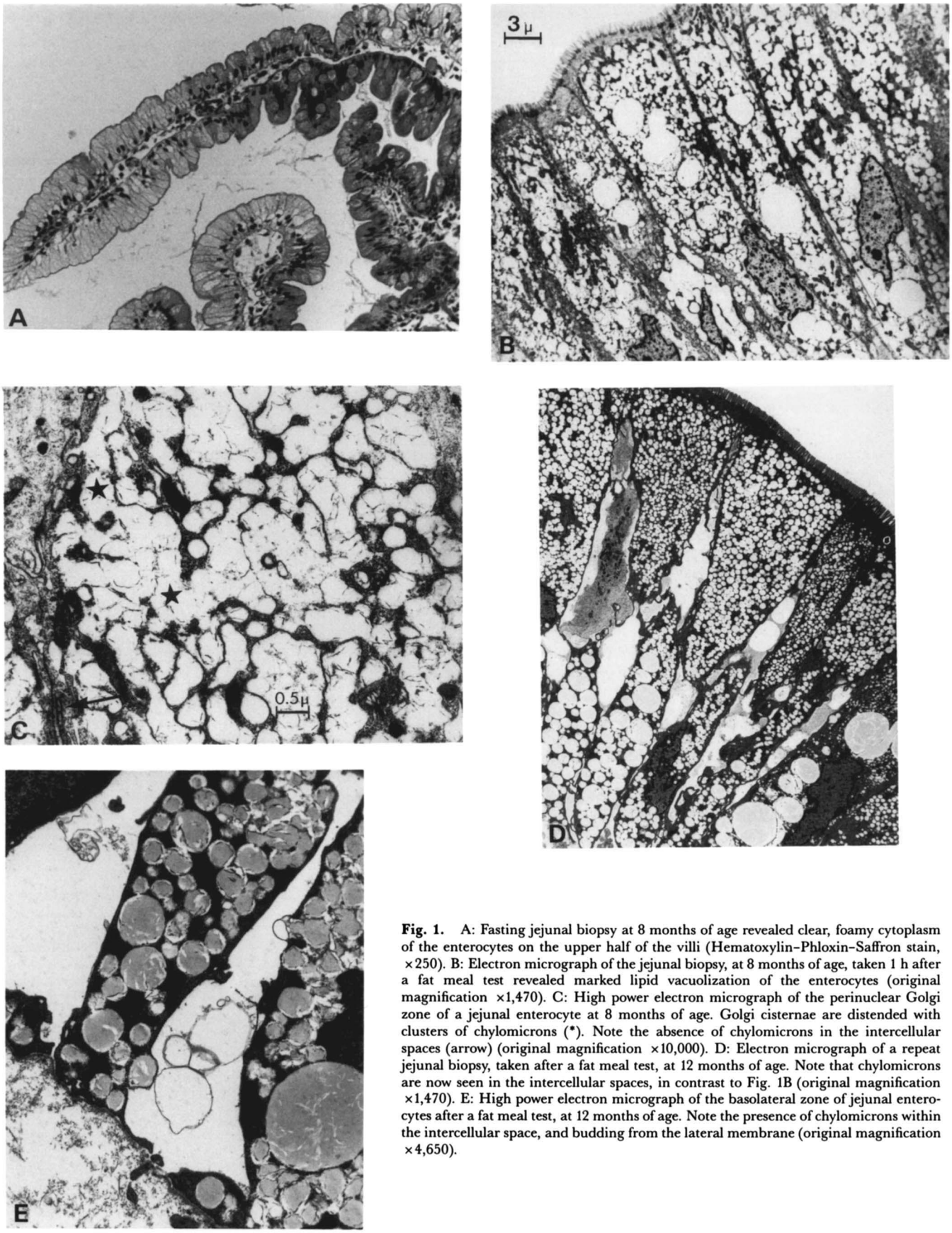

Fig. 1. A: Fasting jejunal biopsy at 8 months of age revealed clear, foamy cytoplasm of the enterocytes on the upper half of the villi (Hematoxylin–Phloxin–Saffron stain, ×250). B: Electron micrograph of the jejunal biopsy, at 8 months of age, taken 1 h after a fat meal test revealed marked lipid vacuolization of the enterocytes (original magnification ×1,470). C: High power electron micrograph of the perinuclear Golgi zone of a jejunal enterocyte at 8 months of age. Golgi cisternae are distended with clusters of chylomicrons (*). Note the absence of chylomicrons in the intercellular spaces (arrow) (original magnification ×10,000). D: Electron micrograph of a repeat jejunal biopsy, taken after a fat meal test, at 12 months of age. Note that chylomicrons are now seen in the intercellular spaces, in contrast to Fig. 1B (original magnification ×1,470). E: High power electron micrograph of the basolateral zone of jejunal enterocytes after a fat meal test, at 12 months of age. Note the presence of chylomicrons within the intercellular space, and budding from the lateral membrane (original magnification ×4,650).

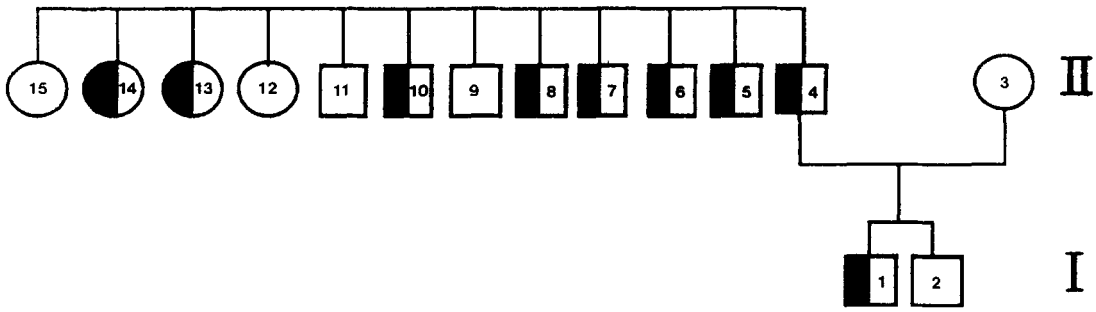

Fig. 2. Pedigree showing the proband and his relatives. The proband is (subject II-1). Subjects with hypocholesterolemia are indicated by a half-shaded circle (female) or square (male). I and II denote the two generations of the proband's family. The concentration of the apolipoproteins and lipoproteins in the plasma for each subject illustrated on this pedigree are listed in Table 1.

lipid-extracted with chloroform–methanol 2:1 (v/v), and the radioactivity of the fractions obtained by thin-layer chromatography (TLC) was measured in a Beckman LS 7500 counter (2, 5). In experiments carried out to measure apoB synthesis, a chylomicron-like fraction was isolated by centrifuging sonicated explants at 25,000 rpm for 30 min and reacted with excess 2D8 monoclonal antibodies (kindly provided by Drs. Ross Milne and Yves Marcel). The immune complex was precipitated by addition of Pansorbin and characterized by SDS-PAGE. Radioactivity corresponding to apoB was determined by liquid scintillation spectrometry.

The studies were approved by the Research Ethics Committee, Ste-Justine Hospital.

RESULTS

The gastrointestinal manifestations encountered in our patient were fairly typical of the features characterizing the hypocholesterolemic syndromes, including abetalipoproteinemia, homozygous hypobetalipoproteinemia, and chylomicron retention disease (1–5). However, the present case met diagnostic criteria for heterozygous hypobetalipoproteinemia, including decreased plasma LDL-cholesterol and detection of a similar lipoprotein profile in first-degree relatives (Table 1, **Fig. 2**). The plasma cholesterol levels were abnormally lower than age-matched controls, and were principally associated with a decrease in the LDL-cholesterol fraction, for both the proband and his father. Their plasma apoB levels were also below normal values. Furthermore, among 14 members of the kinship studied, 8 had the same biochemical abnormalities, and some had abnormally low levels of triglycerides.

In view of the gastrointestinal manifestations in our patient, which were absent in the father despite comparably low apoB levels, we elected to test their response to a fat meal test. As seen in **Table 2,** the increase of triglycerides over fasting values after the ingestion of the fat meal was diminished in the proband at 8 months of age, when compared to the results obtained in control patients. The postprandial triglyceridemia at 2, 3, and 5 h displayed increases of only 28, 32, and 38%, respectively, above basal values, whereas age-matched controls showed higher percentages: 80, 95, and 57%, respectively. At 12 months,

TABLE 2. Plasma triglycerides in response to oral fat load

Subjects	Plasma Triglycerides			
	0 h	2 h	3 h	5 h
		mg/dl		
Proband (8 mo)	100	128 (28)	132 (32)	138 (38)
Proband (12 mo)	112	180 (61)	225 (101)	168 (50)
Father	42	69 (64)	142 (238)	81 (93)
Controls[a]	75 ± 10	135 ± 22 (80)	146 ± 19 (95)	118 ± 18 (57)

After a 12-h fast, patients and controls consumed a fatty meal within 5 min. Blood was taken at the indicated hours and plasma triglycerides were measured. All values are expressed as mg/dl except those in parentheses which represent % increase of triglycerides over fasting values.

[a]Eight normal children (mean ± SEM).

decreased steatorrhea (8%) was accompanied by an improvement of his fat absorption (Table 2). The results of a fat meal test were now comparable to those of controls and to those of his father (Table 2). In keeping with this functional improvement in lipid handling, the repeat biopsy at 12 months of age after a fat load now revealed chylomicrons exiting the basolateral membrane of his enterocytes, entering the intercellular space (Figs. 1D, E).

The composition of the lipoprotein classes obtained by sequential ultracentrifugation is shown in **Table 3.** At 8 months, the patient's VLDL and LDL particles were deficient in TG compared with controls. On the other hand, the HDL fraction was TG-enriched. A decrease of free cholesterol and cholesteryl ester coupled with an increase of phospholipid and protein were noted in the LDL fraction. Therefore, these profiles of lipoproteins differed considerably from those of normolipemic individuals, and the results persisted at 12 months of age, despite improved symptoms. An examination of the proband's VLDL and LDL on SDS-polyacrylamide gel revealed apoB-100, but no truncated species of apoB (**Fig. 3**). This normal apoB profile was also easily detectable in the father's lipoprotein fractions. Furthermore, no apoB variants were detectable in the HDL fraction of our young subject or his father.

In order to examine whether the transient fat malabsorption was caused by a biosynthetic defect of intestinal lipids and apoB, an organ culture technique of his small intestine was used. The results obtained with the jejunal explants provide evidence that the esterification of [^{14}C]oleate into triglycerides, and the incorporation of [^{3}H]leucine into apoB-48 were diminished in the proband, more so at 8 months than at 12 months of age (**Table 4**). Indeed, a trend towards normalization of these parameters was observed in his repeat jejunal biopsy at 12 months, and was similar to results from his father's jejunal explant.

The patient's fat malabsorption has improved significantly over time, with a fecal fat excretion of only 2.2% of intake at age 6. However, supplementation of vitamins A and E have been required, in order to avoid the long term risks of neurological consequences that may not appear until adulthood (6).

TABLE 3. Chemical characterization of lipoproteins

Lipoprotein	TG	CE	FC	PL	PR
VLDL (d 1.006 g/ml)					
Proband (8 mo)	35	12	6	17	31
Proband (12 mo)	41	11	7	16	25
Controls	56	11	8	17	10
LDL (d 1.063 g/ml)					
Proband (8 mo)	1	10	3	43	43
Proband (12 mo)	5	17	4	33	41
Controls	8	32	8	24	27
HDL (d 1.21 g/ml)					
Proband (8 mo)	15	19	2	26	38
Proband (12 mo)	14	18	3	25	40
Controls	5	21	3	24	47

The composition of the proband's and controls' lipoproteins is expressed as percentage of total lipoprotein mass. TG, triglyceride; CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid; PR, protein.

DISCUSSION

Dietary lipids play an important role during the perinatal period as a source of energy and as structural components of cell membranes (12). Triglycerides account for a larger percentage (50–70%) of caloric intake in the neonatal period of many species, compared to later in life (12). Hydrolysis of triglycerides by pancreatic lipase and micellar dispersion of lipolytic products by bile acids assure the intraluminal fat digestion process. However, defective exocrine pancreatic function and failure of the immature liver to deliver qualitative and quantitative bile salts contribute to the malabsorption in the perinatal period which is challenged by a high fat intake (12). In the present case, the fasting jejunal biopsy was engorged with lipid droplets, suggesting that the defect is not primarily intraluminal. The intraluminal factors are therefore unlikely to be solely responsible for the diminished capacity of our patient with hypobetalipoproteinemia to digest and absorb dietary triglycerides. However, the steatorrhea due to deficient intraluminal factors characterizing newborn infants gradually disappears after the first year of life, and may have played a partial role in our patient's unusual presentation early in life. Furthermore, the improved intestinal lipid handling between 8 and 12 months of age may, in part, have resulted from appropriate nutritional management including correction of acute malnutrition as well as lipid-soluble vitamin deficiencies. Other causes of pancreatic exocrine deficiency, such as cystic fibrosis and Schwachman syndrome, were specifically excluded in this case. Furthermore, enteropathies that may present with fat-filled enterocytes, such as milk protein enteropathy, celiac disease, and infectious enteritis, were also eliminated in view of the clinical course, histology, and negative cultures and search for parasites.

Our in vitro results suggest that the intracellular phase of lipid absorption is impaired in symptomatic heterozygous hypobetalipoproteinemia. More specifically, the biosynthesis of triglycerides and apoB was less active at 8 months compared to repeat studies at 12 months. The absence of pathogens, villous atrophy, or inflammatory infiltrates on the biopsy rule out an infectious cause for his transient steatorrhea.

Multiple studies underscore the importance of the addition of a series of proteins to the surface of the lipid droplets for their uptake (1, 3, 6). The presence of apolipoproteins appears to be a prerequisite for the assembly and release of lipoprotein particles (13). Of particular importance for the intestinal synthesis of chylomicrons and VLDL is apoB (14). Its absence, or defect in abetalipopro-

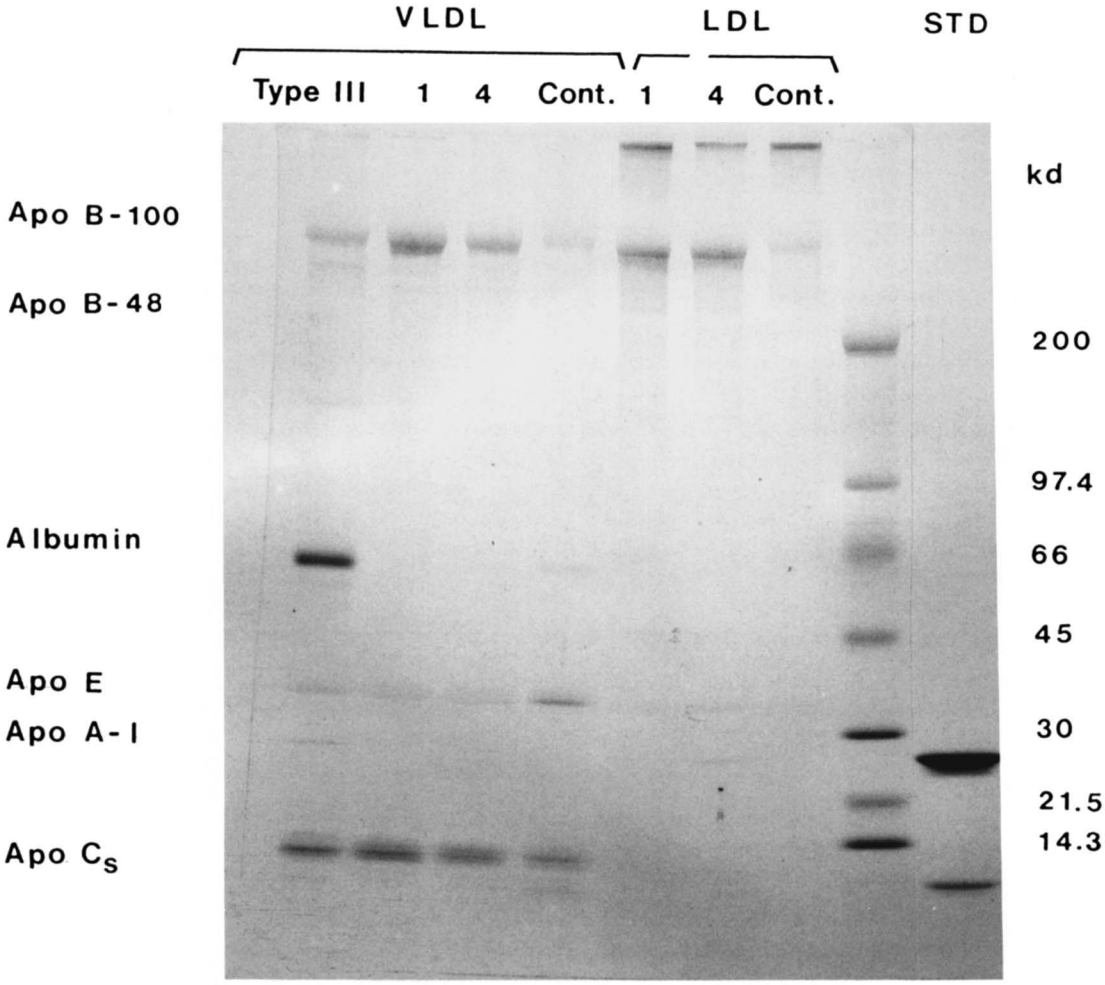

Fig. 3. SDS-PAGE (4–15%) of VLDL and LDL apolipoproteins. The location of apolipoprotein species from the proband (1) and his father (4) was identified by comparison with a healthy subject (cont.) and a familial dysbetalipoproteinemia patient (type III). Molecular weights of standards are indicated to the right of lane STD.

teinemia and homozygous hypobetalipoproteinemia, leads to excessive lipid retention in the small intestine, and to the total absence of delivery of chylomicrons and VLDL (2, 14). Similar abnormalities were observed in the present case. We thus consider that a transient and partial inability to form apoB plays a critical role in the formation and secretion of triglyceride-rich lipoproteins in the symptomatic patient with heterozygous hypobetalipoproteinemia. Further studies are required to understand the ontogeny of apoB synthesis, as well as the factors regulating its expression.

TABLE 4. Synthesis of lipids and apoB in explant cultures of jejunal biopsy specimens

Subjects	Triglycerides	Cholesteryl esters	Phospholipids	ApoB
	dpm/mg of protein of the explants			
Proband				
8 mo	12296	394	15717	20391
12 mo	25720	1872	46253	36676
Father	27983	1915	52512	41596
Controls (n = 2)	34158	3058	77186	48550

Jejunal biopsies were cultured for 18 h in a medium containing [^{14}C]palmitate and [^{3}H]leucine in order to study intestinal lipid and apoB synthesis. The lipid fractions were extracted and quantified as described in Methods. The apoB in chylomicron-like fractions was measured after SDS-PAGE.

Several mutations in the apolipoprotein gene have recently been reported in hypobetalipoproteinemic patients (15–19). They produce shifts in the reading frames of transcription and premature stop codons that direct the synthesis of truncated apoB molecules of varying lengths (20, 21). While nonsense and frameshift mutations in exons 26–29 (coding for the carboxyl-terminal 70% of apoB-100) result in the production of truncated apoB species, mutations occurring in the 5' portion of the apoB gene yield no detectable apoB protein (9). However, when VLDL and LDL fractions were studied by analytical SDS-PAGE, no defects were apparent in the patient's and father's apoB. In addition, we tried to detect the presence

of any truncated forms of apoB in the HDL fraction, in view of the previous identification of abnormal apoB species in HDL. Young and co-workers (22) recently described an apoB-31 fragment that was detectable within the HDL and lipoprotein-deficient serum (LPDS, d > 1.21 g/ml) of plasma. Similarly, McCormick et al. (23) reported that the majority of the truncated apoB-32 was found in HDL and LPDS. In our study, no apoB variants were detectable in our young subject's or his father's HDL fraction. However, we cannot infer that this family's form of familial hypobetalipoproteinemia was not due to a mutation in the apolipoprotein gene. Other investigators have reported that the smallest truncated apoB species described, such as apoB-25 and apoB-29, were not detectable in either the lipoprotein or LPDS fractions, either because of their impaired secretion or their rapid clearance (24, 25). Furthermore, trace levels of truncated apoB species were hardly measurable (15, 17, 22). Finally, a mutation in the 5' portion of the apoB gene yielded no detectable apoB protein (9). Additional studies are therefore needed in order to identify the molecular defect of the apoB gene in this large kindred with hypobetalipoproteinemia.

Other than an apoB mutation, several potential pathophysiological states can potentially explain an association between low levels of cholesterol, LDL-cholesterol, and apoB. In 1987, Vega et al. (26) reported a case of hypobetalipoproteinemia in which plasma apoB was detectable and of normal size. Its low concentration was due to increased hepatic catabolism, reportedly secondary to constitutionally enhanced bile acid synthesis. Fazio et al. (27) have recently published a study on a three-generation family, with several familial hypobetalipoproteinemia members in which linkage analysis showed absence of cosegregation between apoB gene alleles and the hypocholesterolemic phenotype. They concluded that a dominantly transmitted mutation in a gene other than that for apoB is responsible for the low plasma cholesterol levels. We cannot therefore completely exclude this latter possibility in the present kindred.

The differential diagnosis of hypocholesterolemia includes other syndromes in addition to familial hypobetalipoproteinemia, which do not involve the apoB gene. Abetalipoproteinemia is an autosomal recessive disease with absence of apoB and secretion of apoB-containing lipoproteins (1–3). No structural defect in the apoB gene was found (28), and genetic linkage between the apoB gene and abetalipoproteinemia was excluded by restriction fragment length polymorphism analysis (29, 30). Obviously, the microsomal transfer protein that mediates the intracellular transport of membrane-associated lipids and plays a key role in lipoprotein assembly is defective in abetalipoproteinemia (31). As obligate heterozygotes (i.e., parents of probands) have no symptoms and no evidence of reduced plasma lipids, we can easily exclude this disorder as affecting our young patient. Finally, we have recently reported our studies on a disorder known as chylomicron retention disease that has similar clinical and pathological features. The latter condition is associated with a selective inability to secrete apoB from intestinal enterocytes, resulting in fat malabsorption and neurological manifestations (4, 5). The molecular defect is unknown, but appears to be distinct from that of abetalipoproteinemia and familial hypobetalipoproteinemia. As chylomicron retention disease is characterized by chronic malabsorption, unaffected parents, and a different ultrastructural pattern, we do not consider that our young patient is affected by this disorder.

Enormous variability in the levels of apoB have been reported in familial heterozygous hypobetalipoproteinemia (reviewed in ref. 6). There are, furthermore, differences in the symptomatology between individuals with similar levels of circulating apoB. Our proband and his family illustrate this heterogeneity extremely well. Despite apoB levels that were, in fact, similar to those of some of his relatives, our patient presented with clinical malabsorption during infancy. Furthermore, despite a modest increase in apoB level over time, the patient's steatorrhea improved to the normal range. However, his apoB level remains below those of age-matched controls. Indeed, his father had even lower apoB levels, yet had no evidence of malabsorption after a standard fat meal test. Amongst his relatives, several had low apoB levels without hypocholesterolemia. However, these individuals (members 9, 11, and 12) had adult onset diabetes mellitus, which contributes to increased concentrations of lipids and lipoproteins.

The underlying physiologic mechanisms causing the low LDL levels remain to be determined. The reduced concentrations of these apoB-containing lipoproteins can be due either to low secretion rates or to rapid clearance from the plasma. Based on our findings, we suggest that the liver, similar to the intestine, may have abnormally low output of VLDL. However, additional investigation is needed to verify this hypothesis and to clarify the lipoprotein lipase status, which determines the efficiency of the hydrolysis of the VLDL-triglyceride core. Finally, heterozygous hypobetalipoproteinemia represents a heterogenous group of etiologic defects despite overlapping clinical and biochemical findings (6). We cannot exclude the possibility that the patient has another, as yet undefined, cause for his transient malabsorption.

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