

# Normotriglyceridemic Abetalipoproteinemia

## ABSENCE OF THE B-100 APOLIPOPROTEIN

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**ABSTRACT** In the two genetic forms of abetalipoproteinemia described previously, recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia, all lipoproteins that normally contain apolipoprotein B are absent from plasma. We describe here a new disorder in which normal low density and very low density lipoproteins are absent, but in which triglycerides are absorbed from the intestine and chylomicrons are present in plasma. The underlying molecular defect appears to be selective deletion of the hepatogenous B-100 apolipoprotein. The B-48 apolipoprotein found in chylomicrons is spared. These findings suggest that the two species of apolipoprotein B are under separate genetic control and that low density lipoproteins are not normally derived from chylomicrons.

## INTRODUCTION

Two genetic syndromes have been identified in which there is complete absence of beta lipoproteins from plasma (1). In one of these, recessive abetalipoproteinemia, clinical features are manifest only in the homozygous state. In that disorder, normal chylomicrons, very low density lipoproteins (VLDL),<sup>1</sup> and low density lipoproteins (LDL) are absent from plasma. A small amount of an abnormal lipoprotein, which can be concentrated from the low density ultracentrifugal interval, appears to be derived from high density lipoproteins (HDL) (1-4). Clinical features include severe malabsorption of triglycerides (5), acanthocytosis of erythrocytes (often with autohemolysis) (1, 6), de-

ciency of tocopherols and often other fat soluble vitamins in plasma, retinopathy (7), and progressive neurological disease (1). In a genetically distinct disorder, familial hypobetalipoproteinemia, levels of LDL in plasma are approximately half-normal in the heterozygous state with moderate or absent clinical findings. However, a disease essentially indistinguishable from recessive abetalipoproteinemia occurs in the homozygous state (1, 8, 9). Recently, a genetic disorder with severe hypobetalipoproteinemia but with nearly normal absorption of triglycerides has been described (10). In this report we present a distinct, new syndrome, which we term normotriglyceridemic abetalipoproteinemia, in which normal LDL are absent, but in which intestinal absorption of triglycerides and the secretion of chylomicrons into plasma are intact.

Recently, we have demonstrated that the large proteins of human serum lipoproteins termed apolipoprotein B are heterogeneous (11), a finding now shown also for the rat (12). Normal VLDL contain a species with an apparent molecular weight of 549,000, which we denote the B-100 protein in a centile system of nomenclature, whereas the predominant species in chylomicrons has a molecular weight of 246,000 (denoted the B-48 protein). The amino acid compositions of these proteins are distinctly different. The B-48 protein is completely absent from normal human LDL, which contains, in addition to the B-100 protein, two other species, B-74 and B-26, which appear to be complementary fragments of B-100.

The primary molecular defect in the new form of abetalipoproteinemia described here appears to be deletion of the B-100 protein. The ability of the intestine to elaborate the B-48 protein is intact, permitting normal transport of triglyceride from the intestine in chylomicrons. The genetic control of the B-48 protein thus appears to be separate from that of the B-100 species.

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<sup>1</sup> *Abbreviations used in this paper:* apo, apoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; TMU, tetramethyl urea; VLDL, very low density lipoprotein.

## METHODS

**Description of the patient.** The patient was referred at 8 yr of age because she was found to have levels of cholesterol and triglycerides in serum of 25 and 30 mg/dl, respectively. No beta lipoprotein was seen on agarose gel electrophoresis of serum. Lipoproteins with alpha and prebeta mobility were present, however. A chylomicron layer appeared after overnight refrigeration of serum obtained 4 to 8 h after an oral fat load.

In striking contrast with other syndromes of abetalipoproteinemia, this child was obese (Fig. 1). Her weight was above the 97th percentile, and her height was at the 75th percentile. She had alternating esotropia, genu valgum, and a wide-based ataxic gait. She was retarded, with a mental age of 2 to 3 yr. Physical examination, aside from the features noted above, was within normal limits. She does not have the abnormality of hair, dentition, and skin described in a patient with a disorder of beta lipoproteins associated with abnormal tryptophan metabolism (13). Stigmata of autoimmune hypolipidemia were absent. She has a normal 46 XX karyotype. Relatives of this adopted child are not available for study.

Retinal studies, including routine and dark adapted electroretinograms, were normal. In both eyes, B wave amplitudes of 120  $\mu$ V at 22 ms were observed under standard light adapted conditions. After 30 min of dark adaptation, amplitudes of 280 and 240  $\mu$ V were observed (60-ms interval to the second nega-



FIGURE 1 The patient at 8 yr of age (weight, 42.1 kg).

tive peak). The latter responses are in the low normal range (greater than twice the light adapted response). Motor nerve (peroneal) conduction velocity was 53 M/s and sensory nerve (sural) conduction was 50 M/s with normal action potential.

Serum protein electrophoresis and immunoelectrophoresis were normal. Cryoprecipitates were absent and the serum level of the third component of complement was normal (155 mg/dl). Minimal stomatocytic changes were observed in some circulating erythrocytes with no more than 1% acanthocytes present (Fig. 2).

While the patient was eating a typical American diet, her 72-h stool fat excretion was  $\sim$ 10 g. Radiograms of the stomach and duodenum with small bowel follow-through were normal, and a peroral biopsy of the small intestine 16 h after an oral fat load showed normal architecture, with no accumulation of lipid in the terminal villar region, a state compatible with normal chylomicron formation (Fig. 3). There was normal mucin staining with periodic acid-Schiff stain. No residual lipid droplets were seen on a section stained with Oil Red O.

The plasma carotene level was low (31  $\mu$ g/dl) but plasma vitamin A was normal (40  $\mu$ g/dl). Tocopherols were essentially undetectable at 0.1 mg/dl (14). The following studies were all within normal limits: bone age, chest x-rays, electrocardiogram, serum thyroxin (by radioimmunoassay), serum glutamic oxaloacetic transaminase, alkaline phosphatase, lactic dehydrogenase, vitamin B<sub>12</sub>, folic acid, iron, copper, bilirubin, glucose, urea nitrogen, creatinine, uric acid, calcium, phosphorus, sodium, potassium, bicarbonate and chloride, prothrombin time, complete blood count, and urinalysis.

Because of the extremely low levels of tocopherols in plasma, the patient has been given 400 mg/d of *dl* alpha tocopherol. On this treatment, her serum tocopherol levels have been in the normal range, 10–20  $\mu$ g/ml. Over a 4-yr period she has had no new neurological disease, and her ataxia has improved greatly.

**Ultracentrifugal fractionation of serum lipoproteins.** Serum was separated immediately from clotted blood. EDTA 1 mM, (pH 7.4), sodium azide 0.05%, and gentamycin sulfate 0.1 mg/ml were added to serum and to all ultracentrifuge media to prevent hydroperoxidation and bacterial degradation of lipoproteins. Preparation of lipoprotein fractions was begun immediately and was completed without delay. Lipoproteins were separated sequentially by ultracentrifugation in a 40.3 rotor at 12°C in a Beckman model L ultracentrifuge (Beckman

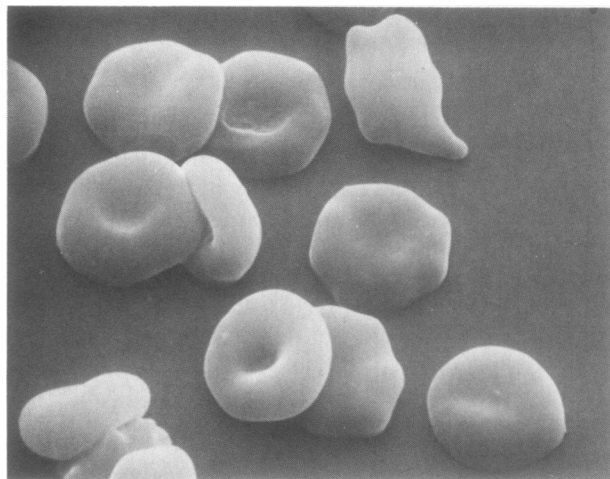


FIGURE 2 Scanning electron photomicrograph of erythrocytes.

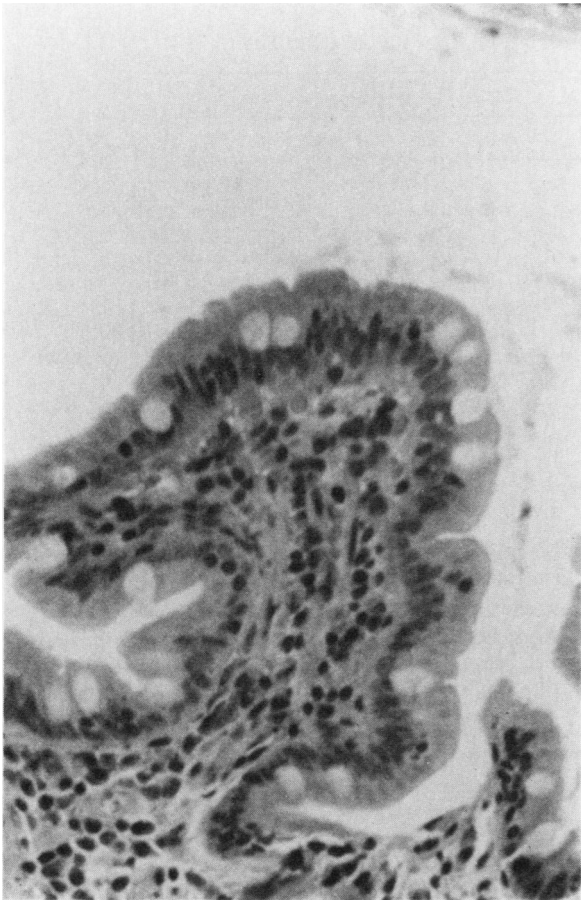


FIGURE 3 Jejunal biopsy taken 16 h after a 100-g fat load—hematoxylin and eosin stain.

Instruments, Palo Alto, Calif.) at the following solvent densities: 1.006, 1.063, and 1.21 g/cm<sup>3</sup> (15). Each lipoprotein fraction was obtained by flotation through an overlayer solution of KBr at the appropriate density and was purified by a second ultracentrifugation at the same density. KBr was removed from fractions by dialysis against 0.15 M saline at 4°C.

**Lipid analyses.** Total cholesterol and triglycerides were measured in serum using the Autoanalyzer II (Technicon Instruments, Tarrytown, N. Y.) (16). Lipids were extracted from dialyzed lipoprotein fractions with 25 vol of 3:1 ethanol:diethyl ether at 25°C for 24 h. Neutral lipid species were measured by the method of Schlierf and Wood (17). Individual classes of phospholipids were separated by the method of Dalal et al. (18) and quantified by determination of lipid phosphorus.

**Lipoprotein analysis.** Serum and lipoprotein fractions were subjected to electrophoresis in agarose gel (19, 21). The content of apolipoprotein B in the lipoprotein fractions was measured by insolubility in 1,1',3,3'-tetramethylurea (TMU) (20). The content of TMU-soluble apoproteins was determined by electrophoresis in polyacrylamide gels (20), and the isoforms of apoprotein (apo) E were quantified by isoelectric focussing (21). A comparison of the immunoreactivity of the patient's serum and lipoprotein fractions with that of normal serum was made by double immunodiffusion (22), using goat antiserum to whole human apo LDL. A series of dilutions of each sample was examined and a semi-quantitative estimate of

equivalence was determined from the respective dilutions which gave precipitin arcs of similar intensity. The contents of proline-rich apolipoprotein (23), apolipoprotein A-I (24), and apolipoprotein D (25) in whole serum were determined immunochemically. Lp(a) specific antigen was detected by double immunodiffusion. Structure of the lipoproteins of the three density intervals was studied by electron microscopy of preparations negatively stained with 2% potassium phosphotungstate (26). The flotation properties of high density lipoproteins were examined by analytical ultracentrifugation (27).

Apolipoprotein B-like protein was separated from the other apolipoproteins of the  $d < 1.006$  g/cm<sup>3</sup> lipoproteins with 4.2 M TMU (20). Lipids were removed from the precipitate by two extractions with 3:1 ethanol:diethyl ether (vol/vol) for 18 h at 23°C. For comparison of the apparent molecular weight of this protein with the known species of apo B of normal human serum, it was dissolved in 50 mM sodium phosphate (pH 7.2) containing 10 mg sodium dodecyl sulfate (SDS)/mg of protein, and 1% 2-mercaptoethanol (both from Pierce Chemical Co., Rockford, Ill.) and subjected to electrophoresis in SDS in 3% polyacrylamide gel (11). The single apo B element present was purified by preparative electrophoresis in SDS (28). The protein was precipitated from the eluate with 20% TCA. After centrifugation (15,000 g · min), the TCA solution was decanted, and the precipitate was washed twice with acetone to remove traces of TCA. Triplicate samples of the precipitated protein were hydrolyzed in deaerated 6 N HCL (Ultrex, J. T. Baker Chemical Co., Phillipsburg, N. J.) at 105°C at each of three time intervals (22, 72, and 120 h). Amino acid compositions were determined using a two-column program on a Beckman model 121 M amino acid analyzer (Beckman Instruments) (29). Contents of labile amino acids (serine and threonine) were calculated by extrapolation to zero time, and those of isoleucine, leucine, and valine were calculated from the 120-h values. Contents of half-cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, by the method of Hirs (30).

**Studies of lipid transport.** Serum lipoproteins were studied after the patient fasted for 16 h and after she ingested fat meals containing 100 g of olive oil suspended in ice-milk. The contents of cholesterol, triglycerides, and unesterified fatty acids (31) were measured periodically over 12 h following the fat meals. To determine whether the patient could produce triglyceride-rich lipoproteins in the absence of alimentary triglyceride, serum lipoproteins were analyzed after she had been eating a fat-free formula (providing 120% of her estimated daily caloric requirement) for 5 d. Post-heparin lipolytic activity of plasma was measured by the method of Boberg and Carlson (32), following injection of 0.2 mg heparin/kg body wt.

## RESULTS

**Chemical composition of lipoproteins: response to dietary fat.** No lipoprotein band of beta mobility has been present in electrophoretograms of the patient's serum either after a 14-h fast or at any time after a fat-rich test meal. Within 2 h after ingesting the 100-g olive oil formula, the patient's serum triglycerides increased from 25–30 mg/dl to as high as 240 mg/dl, and returned gradually to basal levels over ~6 h. After a 100-g fat load, plasma levels of unesterified fatty acids decreased from a basal level of 1.17 meq/liter to 0.63 meq/liter at 4 h and 0.58 meq/liter at 7 h. Whenever serum triglyceride levels increased to 200 mg/dl or more after fat meals,

TABLE I  
Content and Composition of Serum Lipoproteins 4 h after an Oral Fat Load\*

| Ultracentrifugal fraction | Total mass   | Composition |     |      |      |     |      |
|---------------------------|--------------|-------------|-----|------|------|-----|------|
|                           |              | PL          | FC  | CE   | TG   | X   | Prot |
| <i>g/cm<sup>3</sup></i>   | <i>mg/dl</i> | %           |     |      |      |     |      |
| <i>d</i> < 1.006          | 96           | 9.2         | 3.6 | 5.3  | 70.4 | 4.0 | 7.8  |
| 1.006 < <i>d</i> < 1.063  | 1.0          | 22.4        | 4.0 | 44.0 | 7.2  | 2.4 | 20.0 |
| 1.063 < <i>d</i> < 1.21   | 74           | 18.1        | 2.6 | 21.4 | 2.0  | 2.8 | 53.1 |

PL, total phospholipid; FC, unesterified cholesterol; TG, triglycerides; Prot, protein; X, unidentified polar lipid typical of hydroperoxidized olefinic lipids.

\* 100 g of olive oil suspended in 50 g of ice milk.

overnight refrigeration of serum samples obtained between 2 and 5 h after the meal revealed a narrow but distinct white supernatant lipoprotein layer as seen in normal postprandial chylomicronemia. Contents of protein and of the major classes of lipids in lipoproteins separated ultracentrifugally from serum drawn 4 h after a 100-g oral fat load are shown in Table I. The percent content of amphipathic lipids and protein in the triglyceride-rich lipoproteins in this preparation would be appropriate for pseudomicellar particles 600 Å in diameter, assuming a surface monolayer of 21 Å thickness (33). Each lipoprotein fraction contained abnormal polar lipids, which are presumed to be derived from olefinic lipids by hydroperoxidation. The chemical composition of lipoproteins from fasting serum was similar to that in Table I, except that the total mass of lipoproteins in the *d* < 1.006 *g/cm<sup>3</sup>* fraction was about 25 mg/dl. After a carbohydrate-rich, fat-free diet was fed for 5 d, the triglyceride level in serum had increased to 76 mg/dl. There was no difference in the masses of HDL or LDL, calculated as the sums of the constituent lipids and proteins in the respective ultracentrifugal intervals, between serum drawn after a 14-h fast and that drawn during or following postprandial lipemia.

The detailed composition of phospholipids in the three ultracentrifugally separated lipoprotein fractions described in Table I are shown in Table II. In each fraction the percent content of sphingomyelin is increased above normal at the expense of phosphatidylcholine as described in recessive abetalipoproteinemia (2) and homozygous hypobetalipoproteinemia (34). The post-heparin lipolytic activity of plasma drawn after a 14-h fast was only 0.046 μmol/ml per min, compared with that of a normal control of 0.21 μmol/ml per min. In the presence of 1 M NaCl, the lipolytic activity of the patient's plasma was 0.038 μmol/ml per min.

*Electron microscopic appearance of lipoproteins.* Electron photomicrographs of the lipoproteins in the ultracentrifugal fractions of the patient's serum are shown in Fig. 4. The lipoproteins of the HDL interval are spheres with a modal diameter of ~80 Å. Few particles of the diameter of HDL<sub>2</sub> are observed. This finding was supported by the absence of lipoproteins with hydrodynamic characteristics of HDL<sub>2</sub> upon analytical ultracentrifugation. The lipoproteins of the LDL interval appear to be of two types. The predominant particles are spheres of ~300 Å diam, resembling VLDL rem-

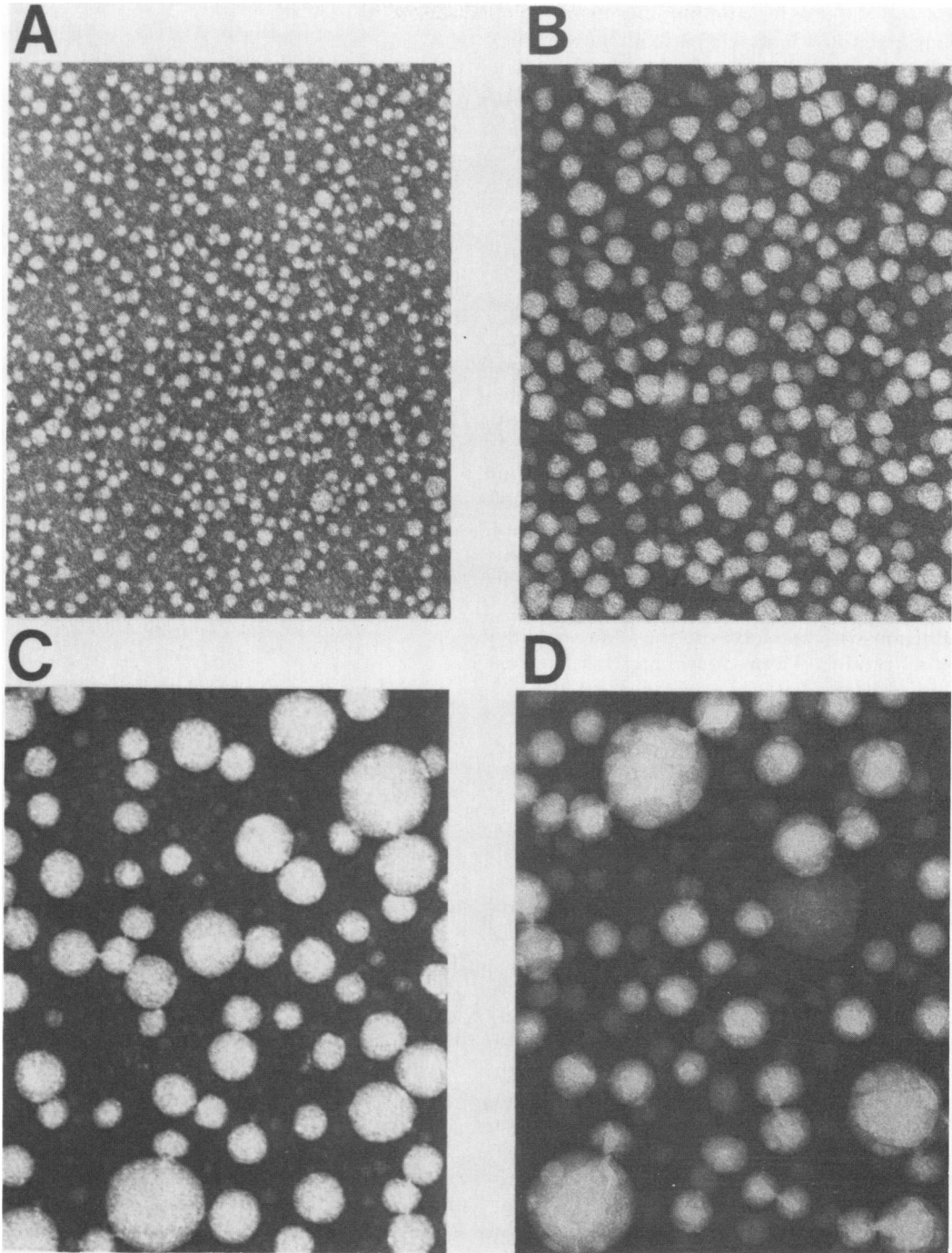
TABLE II  
Composition of Phospholipids

| Ultracentrifugal fraction | PC  | LYS | SPH | INOS | SER    | ETH | OTHER  |
|---------------------------|-----|-----|-----|------|--------|-----|--------|
| %                         |     |     |     |      |        |     |        |
| <i>d</i> < 1.006          | 55  | 8   | 28  | 2    | 0.5    | 4   | 2.5    |
| 1.006 < <i>d</i> < 1.063  | 65  | 3   | 27  |      | --1--* |     | --4--* |
| 1.063 < <i>d</i> < 1.21   | 60‡ | 3   | 30‡ | 2.6  | 1      | 3   | 0.4    |

PC, phosphatidylcholine; LYS, lysophosphatidyl choline; SPH, sphingolipids; INOS, phosphatidyl inositol; SER, phosphatidyl serine; ETH, phosphatidyl ethanolamine; OTHER, polar phospholipids presumed to be hydroperoxidized.

\* Total content was insufficient for separate measurement of the minor constituents of the LDL interval.

‡ For comparison, PC was found to constitute 53%, and SPH 41.7% of the HDL phospholipids in abetalipoproteinemia; PC was 70.7%, and SPH 13.8% of phospholipid in normal HDL (3).



**FIGURE 4** Electron photomicrographs of serum lipoproteins, negatively stained with 2% potassium phosphotungstate. A, HDL; B, LDL ( $1.006 < d < 1.063 \text{ g/cm}^3$ ); C,  $d < 1.006 \text{ g/cm}^3$  lipoproteins from serum drawn 2 h after an oral fat load; D,  $d < 1.006 \text{ g/cm}^3$  after 5 d on a carbohydrate-rich, fat-free formula diet. All are at 180,000-fold magnification.

nants. The other particles,  $\sim 200 \text{ \AA}$  diam, are somewhat cuboidal in shape. The lipoproteins of the  $d < 1.006 \text{ g/cm}^3$  fraction are spherical, with diameters of 300–

1,000  $\text{\AA}$  during postprandial lipemia as well as in serum drawn after an overnight fast.

*Soluble apolipoproteins.* Quantitative electro-

phoresis of the TMU-soluble apolipoproteins of the  $d < 1.006 \text{ g/cm}^3$  fraction 4 h after a fat load showed the following distribution (as percentage of total TMU-soluble protein): apo C-I, 11.9%; apo E, 16.2%; apo C-II, 17%; apo C-III<sub>0</sub>, 3%; apo C-III<sub>1</sub>, 16%; apo C-III<sub>2</sub>, 30%; apo C-III<sub>3</sub>, 7%. Gel electrophoretograms obtained in the same system with HDL showed apo A-I, apo A-II, apo D, and apo C-I in proportions similar to those in normal HDL. As with normal HDL<sub>3</sub>, the contents of apo C-II and apo C-III appeared lower than in normal whole HDL. Isoelectric focussing again showed low levels of apo C-III<sub>1</sub>. The ratio of the E<sub>3</sub> to E<sub>2</sub> isoforms was 1.56 unreduced by 2-mercaptoethanol, and 1.31 after reduction, indicating the presence of a normal E<sub>3</sub> genotype (35). Analysis by radioimmunoassay of the total apolipoprotein A-I in serum, drawn 5 h after a fat meal, yielded a value of 95.6 mg/dl, compared with a value of 117.0 mg/dl for a pooled normal adult serum sample. The apo A-I content of the patient's  $d < 1.006 \text{ g/cm}^3$  fraction was only 0.5 mg/dl. The proline-rich apolipoprotein was present in whole serum at a concentration of 34 mg/dl (medium normal 20 mg/dl). The apo D content of whole serum was 1.93 mg/dl (normal range 4.5–5.5 mg/dl). Immunodiffusion with antiserum to the Lp(a) specific apolipoprotein produced a precipitin arc with the patient's whole serum.

**Characterization of apolipoprotein B.** By immunodiffusion of serial dilutions of the patient's whole serum, reactivity with antibodies to LDL was equivalent to a concentration of normal LDL apo B of only 0.2 mg/dl, despite the fact that the patient's lipoproteins contained 7.5 mg/dl of TMU-insoluble protein (37% of the protein in the  $d < 1.006 \text{ g/cm}^3$  lipoprotein fraction). After delipidation with 3:1 ethanol:ether, the protein precipitated by TMU, unlike apo B from normal LDL, could be completely dissolved in 8 M urea.

The mobility of the TMU-insoluble protein from the patient's  $d < 1.006 \text{ g/cm}^3$  lipoprotein fraction in SDS-gel electrophoretograms is shown in Fig. 5. The predominant protein is present as a narrow band with mobility identical to that of the B-48 protein of human chylomicrons. The B-100 protein normally found in VLDL and LDL is absent, as are the B-74 and B-26 proteins normally found in LDL. The minor band in the lower portion of the gel appears to correspond to one of the elements of lower molecular weight found in chylomicrons. (This material has not been isolated in sufficient amounts for analysis.) The apparent molecular weight of the predominant TMU-insoluble protein in the patient's lipoproteins was estimated by its volume of elution from the preparative SDS-gel electrophoresis apparatus as shown in Fig. 6. Its elution volume was identical to that of the B-48 protein derived from human thoracic duct chylomicrons. This corresponds to an apparent molecular weight of  $264,000 \pm 8,150$ .

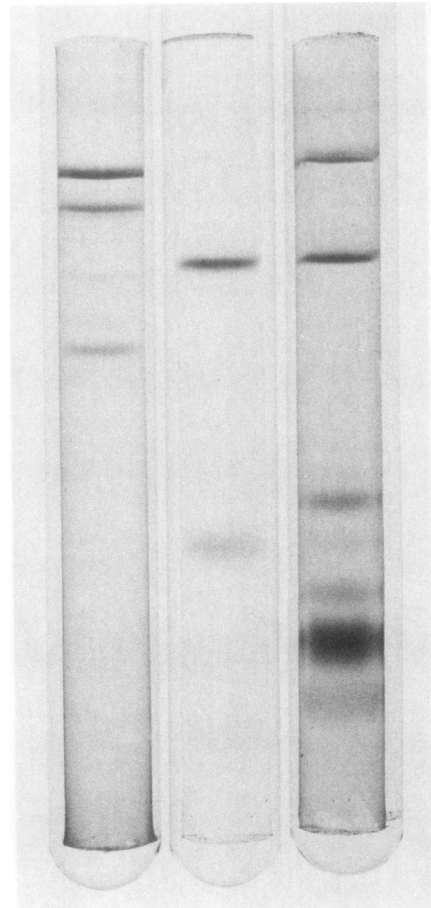


FIGURE 5 Comparisons of the B apolipoproteins of the patient's lipoproteins with those of normal serum lipoproteins, by SDS electrophoresis in 3% polyacrylamide gels. Left: apo B from normal LDL. The three principal protein bands are (top) B-100, (middle) B-74, (lower) B-26. Center: apoproteins from the  $d < 1.006 \text{ g/cm}^3$  fraction of the patient's serum lipoproteins. Right: total apolipoprotein fraction from normal human thoracic duct chylomicrons. The uppermost band is the B-100 protein, next below is the B-48 protein, and below are soluble apolipoproteins, including the proline-rich apoprotein, apo E, apo A-I, and C-apolipoproteins.

The amino acid compositions of two preparations of the patient's 264,000 mol wt component, isolated by preparative SDS-gel electrophoresis, are shown in Table III. The contents of many of the amino acids do not differ greatly between the hepatogenous B-100 and enterogenous B-48 proteins. However, in those cases where distinct differences are apparent (glycine, alanine, methionine, isoleucine, and phenylalanine), the content in the patient's protein is close to that of the normal B-48 protein.

Despite the increase of triglycerides in plasma which occurred during carbohydrate feeding, the SDS-gel pattern of the patient's apo B remained unchanged.

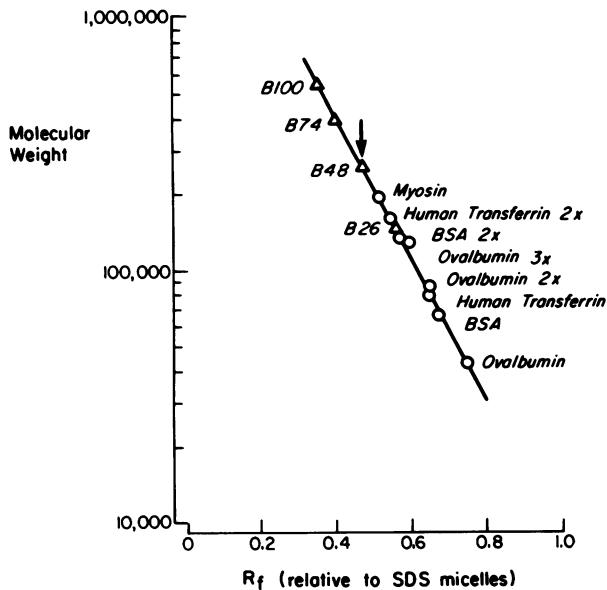


FIGURE 6 SDS-gel electrophoresis of B apolipoproteins. Arrow indicates the elution volume of the B protein prepared from the patient's  $d < 1.006 \text{ g/cm}^3$  lipoproteins. B-100, B-74, and B-26 proteins were obtained from normal LDL; B-48 protein was obtained from human thoracic duct chylomicrons. Other proteins are purified standards; 2 $\times$ , 3 $\times$  signify putative oligomers of the standard proteins.

## DISCUSSION

The syndrome described here differs from the two forms of abetalipoproteinemia reported previously (1, 36) in that essentially normal levels of triglycerides are present in plasma, and intestinal absorption of triglycerides is normal. Also, in contrast to the syndrome of severe hypobetalipoproteinemia with normal absorption of triglycerides described by Steinberg et al. (10), our patient's serum is devoid of normal LDL. Despite the presence of triglyceride-rich lipoproteins in plasma, the syndrome which we describe here shares a number of features with the other two forms of abetalipoproteinemia, recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia. In both, the ratio of sphingomyelin to phosphatidylcholine in plasma is increased (2, 34). In the disorder we describe, this phenomenon is evident in the  $d < 1.006 \text{ g/cm}^3$  lipoprotein fraction as well as in the HDL. As in recessive abetalipoproteinemia (1, 4), homozygous hypobetalipoproteinemia (1), and the syndrome described by Steinberg et al. (10), the lipoproteins are deficient in the monosialated form of apo C-III. This observation is consistent with the hypothesis, based on studies of inhibition of VLDL secretion in the rat with orotic acid (37), that apo C-III<sub>1</sub> is primarily secreted from the liver with VLDL, whereas apo C-III<sub>2</sub> is secreted with HDL. Levels of total HDL are decreased in this syndrome, a

finding common to the three disorders mentioned above. In contrast to recessive abetalipoproteinemia, however, where levels of lipoproteins with flotation properties of HDL<sub>2</sub> are nearly normal but the HDL<sub>3</sub> fraction is markedly decreased (2, 4, 38), HDL<sub>2</sub> lipoproteins are virtually absent and levels of HDL<sub>3</sub> are low in normotriglyceridemic abetalipoproteinemia. The low level of apo D we have observed in plasma in this disorder may be related to the abnormalities of HDL. Post-heparin lipolytic activity has been found to be low in recessive abetalipoproteinemia (39, 40). It has been postulated that this might reflect lack of induction due to deficiency of triglyceride-bearing lipoproteins in plasma. The low levels of activity we have observed in normotriglyceridemic abetalipoproteinemia in the presence of normal secretion of chylomicrons, suggests that some other factor is responsible. Certainly this is not due to deficiency of apo C-II cofactor because the contents of that protein which we have observed in the  $d < 1.006 \text{ g/cm}^3$  lipoprotein fraction and in HDL considerably exceed the levels which appear to be required for activation of the enzyme (40). As in recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia, there is an extremely small amount of lipoprotein material in the LDL ultracentrifugal interval. The electron micrographic appearance of lipoproteins concentrated from this fraction suggests they are of two types. Spherical particles,  $\sim 300 \text{ \AA}$  diam, are probably remnants of the patient's triglyceride-rich lipoproteins, formed by intravascular lipolysis. The smaller, somewhat cuboidal particles are probably analogous to the rectangular lipoproteins isolated by Forte and Nichols (42) and Kayden (36) from this ultracentrifugal fraction of the serum of patients with recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia.

The syndrome we describe shares few of the clinical features of recessive abetalipoproteinemia and homozygous hypobetalipoproteinemia. Nearly normal content of triglycerides in stool, lack of retention of lipid in intestinal epithelial cells, and normal lipemia following an oral fat load indicate that absorption of triglycerides and secretion of chylomicrons proceeds normally. The patient's obesity, contrasting markedly with the depleted adipose mass characteristic of other forms of abetalipoproteinemia, attests to the efficiency of intestinal triglyceride transport in this disorder. To date, function and appearance of the retinas remain normal, though dark adapted retinograms show a response at the lower limit of normal. The only detectable neurological deficits are ataxia, mental retardation, and alternating esotropia. The rarity of acanthocytes in the patient's blood suggests that the presence of triglyceride-bearing lipoproteins may preserve normal morphology of erythrocytes. The fact that the sphingo-

**TABLE III**  
*Comparison of the Amino Acid Composition of Apolipoprotein B from Normotriglyceridemic Abetalipoproteinemia with Normal Human Apo B-48 and Apo B-100*

| Amino acid | B-48 from normal human chylomicrons | B-48 from normotriglyceridemic abetalipoproteinemia |         |        | B-100 from normal LDL | SEM  |
|------------|-------------------------------------|---|---------|--------|-----------------------|------|
|            |                                     | Prep 1†   | Prep 2† | Mean   |                       |      |
|            |                                     | <i>mol/10<sup>3</sup> mol</i>                       |         |        |                       |      |
| Lys        | 77.51*                              | 79.72   | 77.08   | 78.40  | 79.85*                | 0.75 |
| His        | 24.54                               | 25.31   | 26.51   | 25.91  | 25.70                 | 0.51 |
| Arg        | 38.34                               | 35.32   | 37.79   | 36.56  | 33.77                 | 0.47 |
| Asp        | 107.08                              | 107.83  | 110.00  | 108.92 | 106.56                | 0.82 |
| Thr        | 68.33                               | 65.95   | 67.65   | 66.80  | 66.44                 | 1.08 |
| Ser        | 90.51                               | 87.22   | 88.07   | 87.65  | 86.43                 | 1.80 |
| Glu        | 110.32                              | 103.72  | 109.61  | 106.67 | 116.16                | 0.88 |
| Pro        | 38.71                               | 37.80   | 37.29   | 37.55  | 38.54                 | 0.47 |
| Gly        | 56.13                               | 58.10   | 55.64   | 56.87  | 47.31                 | 0.42 |
| Ala        | 65.76                               | 65.92   | 65.16   | 65.54  | 60.50                 | 0.35 |
| ½Cys       | 6.38                                | 5.40  | 5.59    | 5.50   | 4.45                  | 0.11 |
| Val        | 52.60                               | 53.90   | 51.66   | 52.78  | 55.60                 | 0.45 |
| Met        | 21.25                               | 20.41   | 21.15   | 20.78  | 15.95                 | 0.16 |
| Ile        | 46.72                               | 52.32   | 50.64   | 51.48  | 60.27                 | 0.57 |
| Leu        | 121.32                              | 124.73  | 121.40  | 123.07 | 118.46                | 1.18 |
| Tyr        | 33.79                               | 35.72   | 33.97   | 34.85  | 33.58                 | 0.24 |
| Phe        | 40.76                               | 40.63   | 40.77   | 40.70  | 50.46                 | 0.25 |

\* Means of data on three preparations, each of which represents triplicate analyses at each of three different times of hydrolysis (22, 72, and 120 h). SEM derived from pooled estimates of variance over 100 consecutive analyses is provided as an index of precision of amino acid analysis.

† Each preparation was analyzed in triplicate at three time points.

myelin-to-phosphatidylcholine ratios in plasma lipoproteins are similar to those found in the two previously recognized forms of abetalipoproteinemia suggests that some additional factor may be responsible for acanthocytosis.

Studies of the patient's apolipoproteins indicate that a categorical deficiency of one of the principal species of apo B is the underlying biochemical defect in this disorder. No B-100 protein is detectable in the patient's plasma. The greatly diminished immunoreactivity of the patient's lipoproteins with antibodies to LDL is consistent with our observation that cross-reactivity of human intestinal B-48 protein with antisera against the B-100 protein is limited (43). This is supported by similar findings with rat apo B (12). The B apoprotein present in the patient's serum has the same apparent molecular weight (264,000) as does normal human intestinal B-48 protein, whereas that of the B-100 species is 549,000. The absence of the B-74 and B-26 proteins, which are probably complementary fragments of the B-100 molecule, further underscores the categorical deletion of the B-100 protein. Also, the amino acid composition of the patient's apo B is similar to that of the B-48 protein. Thus, it appears that the intestinal apolipoprotein B-48 is completely spared in this disorder, allowing the secretion and peripheral metab-

olism of chylomicrons to proceed normally. This suggests that the B-100 and B-48 proteins are under separate genetic control. The absence of B-100 protein in the patient's serum indicates that she must be homozygous for deletion of that protein. Studies of her family members would be important in evaluating this hypothesis because both parents would thus be obligate heterozygotes for the deletion. Unfortunately, none of the patient's relatives have been found for study.

The reactivity of B-48 protein with antibodies raised against apo B of LDL indicates that these proteins contain a common subunit. However, the observation that the immunoreactivity of B-48 with these antisera is considerably less than that of B-100 suggests that B-48 contains subunit protein which does not occur in the B-100 protein. Perhaps B-100, in parallel fashion, may have subunits which do not occur in the B-48 protein. Absence of such organ-specific subunits in the B-100 protein, based on a structural gene defect, could account for the disorder we describe. According to this model, deletion of the common subunit would result in absence of apo B-containing lipoproteins of both hepatic and intestinal origin. If this model obtains, then an additional disorder could exist in which intestinal organ-specific subunits of apo B are lacking. This would result in absence of the B-48 protein and failure of se-



cretion of chylomicrons, but secretion and metabolism of B-100, and hence of VLDL and LDL, would be normal. Whereas familial hypobetalipoproteinemia would be consistent with a structural gene defect, recessive abetalipoproteinemia might more likely result from other genetic defects, such as abnormalities in post-translational modifications of apo B, polymerization of subunits of apo B, a regulator gene, transport of apo B, or secretion of lipoproteins from cells. Abnormally rapid catabolism of lipoproteins containing apo B could also cause abetalipoproteinemia. In addition, it is possible that the structural genes for components of B-48 and B-100 are close to one another on the same chromosome, thus tending to be deleted together. This would result in absence of all apo B-containing lipoproteins as is the case in homozygous hypobetalipoproteinemia.

The deletion of the apo B-100-containing species of lipoproteins in this disorder affords a unique opportunity to study the metabolism of triglyceride-rich lipoproteins of intestinal origin. The absence of normal LDL indicates that, unlike VLDL, chylomicrons are not normally converted to LDL. The small amount of intermediate density lipoprotein present suggests that only some chylomicron remnants enter the intermediate density interval or that they are removed very rapidly from plasma. The observation that levels of triglycerides in the patient's plasma increase threefold during 5 d of feeding a fat-free hypercaloric diet is not readily explained without further studies. It is possible that the liver is capable of producing the B-48 protein when the production of B-100 is blocked, and hence that the increase of triglycerides in plasma could reflect the presence of abnormal hepatogenous VLDL. Other possibilities are that the patient's intestine may be the site of production of the triglyceride-rich particles following conversion of the carbohydrate to fatty acids *in situ*, or that removal of triglyceride-rich lipoproteins from plasma is impeded during carbohydrate loading.

The extremely low levels of tocopherol in the patient's plasma before supplementation was given indicate that there is a defect in tocopherol transport despite the apparently normal formation of chylomicrons, presumably reflecting a requirement for VLDL or LDL. The observation that levels of tocopherols in the patient's plasma increased to normal during supplementation with 400 mg *dl* alpha tocopherol/d indicates that if a defect in absorption of tocopherol is present, it can be overcome by increasing intake of the vitamin. At the same time, the striking coincident improvement of the patient's ataxia suggests that this neurological defect may have been due to tocopherol deficiency. This experience is consistent with observations of amelioration or stabilization of neurological and retinal manifestations of abetalipoproteinemia during treatment with tocopherols (44, 45). Normal levels of vitamin A in

serum and normal prothrombin times suggest that transport of vitamin A and vitamin K are normal in normotriglyceridemic abetalipoproteinemia.

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