Familial Apolipoprotein E Deficiency

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Abstract

A unique kindred with premature cardiovascular disease, tubo-eruptive xanthomas, and type III hyperlipoproteinemia (HLP) associated with familial apolipoprotein (apo) E deficiency was examined. Homozygotes (n = 4) had marked increases in cholesterol-rich very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), which could be effectively lowered with diet and medication (niacin, clofibrate). Homozygotes had only trace amounts of plasma apoE, and accumulations of apoB-48 and apoA-IV in VLDL, IDL, and low density lipoproteins. Radioiodinated VLDL apoB and apoE kinetic studies revealed that the homozygous proband had markedly retarded fractional catabolism of VLDL apoB-100, apoB-48, and plasma apoE, as well as an extremely low apoE synthesis rate as compared to normals. Obligate heterozygotes (n = 10) generally had normal plasma lipids and mean plasma apoE concentrations that were 42% of normal. The data indicate that homozygous familial apoE deficiency is a cause of type III HLP, is associated with markedly decreased apoE production, and that apoE is essential for the normal catabolism of triglyceride-rich lipoprotein constituents.

Introduction

Type III hyperlipoproteinemia (HLP)[1] is an uncommon lipid disorder characterized by elevations of both plasma cholesterol and triglyceride due to an increase in chylomicron remnants, very low density lipoproteins (VLDL), and intermediate density lipoproteins (IDL) (1–3). These lipoproteins in type III HLP are cholesterol rich, and migrate to an abnormal beta position on electrophoresis (4). Type III HLP patients generally present in adulthood, often develop premature coronary artery disease and peripheral vascular disease, and may have palmar and tubo-eruptive xanthomas (1–3). Type III HLP has been associated with abnormalities of apolipoprotein (apo) E (5–12).

ApoE is a glycoprotein of 34,000 kD, which serves as a constituent of plasma triglyceride-rich lipoproteins and high density lipoproteins (HDL) (7). Human apoE is a polymorphic protein having three major isoforms separated by isoelectric focusing (apoE2, E3, and E4) (5, 6). Each of the known polymorphic forms of apoE is coded by a separate allele, and those alleles are inherited in a codominant fashion at a single genetic locus (6). The primary amino acid sequence of apoE has been reported, and it has been shown that apoE4 differs from apoE3 by an arginine for cysteine substitution at residue 112, while apoE2 differs from apoE3 by a cysteine for arginine substitution at residue 158 (7, 8). ApoE3 is the most common isoform present in the general population, may be associated with normal plasma lipids, and has been proposed to be the normal allele (5, 6). An increased prevalence of the apoE4 phenotype has been observed in patients with type V hyperlipoproteinemia (13). More recently, the apoE4 allele has been associated with elevated LDL cholesterol levels in plasma (14–16). Type III HLP has been associated with apoE2 homozygosity, various rare mutations of apoE, and apoE deficiency (5–12).

The purpose of the present study was to define the clinical, genetic, biochemical, and metabolic characteristics of kindred members in the first reported apolipoprotein E deficiency kindred (12).

Methods

Clinical data. The proband for the apoE-deficient kindred was a 60-year-old Black female of 161 cm height, 69.8 kg, with a 10-year history of small tubo-eruptive xanthomas on her elbows and knees, a 5-yr history of hypertension, a history of intermittent cigarette smoking, and a 3-yr history of chest pain consistent with angina pectoris. The patient underwent a hysterectomy at age 50 yr for fibroid tumors. Coronary angiography at age 60 yr revealed an 80% narrowing of the first diagonal branch of the left anterior descending coronary artery; other coronary arteries had no detectable narrowing. Routine laboratory data including complete blood count, chemistries, thyroid, liver, and kidney function tests were within normal limits (except for plasma lipids). Plasma lipid and lipoprotein cholesterol data are given in Table I. Plasma concentrations of vitamins A and E as measured by high pressure liquid chromatography, and plasma fatty acid composition as determined by gas liquid chromatography were within normal limits. Physical examination was unremarkable except for small tubo-eruptive xanthomas on the elbows and knees, and arcus senilis. No corneal opacification, lymphadenopathy, or hepatosplenomegaly was noted.

An initial attempt at transluminal coronary angioplasty was unsuccessful. The patient sustained a subendocardial myocardial infarction ~3 mo thereafter. A second angioplasty was successful in reducing the area of stenosis by at least 50%. Adequate blood pressure control was achieved with propranolol 40 mg per os (p.o.), three times daily. A low fat (25% of calories), low cholesterol (<250 mg/d), high polyunsaturated/

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Received for publication 26 June 1985 and in revised form 15 July 1986.

1. *Abbreviations used in this paper:* apo, apolipoprotein; CAD, coronary artery disease; HLP, hyperlipoproteinemia; PAGE, polyacrylamide gel electrophoresis.

J. Clin. Invest.

0021-9738/86/11/1206/14 $1.00
Volume 78, November 1986, 1206–1219

Table I. Plasma Lipid and Lipoprotein Cholesterol Concentrations

Subject	Age	Sex	Plasma		Cholesterol			VLDL C/TG ratio	ApoE phenotype
			Cholesterol	Triglyceride	VLDL	LDL	HDL		
			mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*		
Family 1									
Sibling 1	45	M	614*	294*	381*	200‡	33	1.30	E0
Offspring 1	11	M	228*	99	29*	146*	53	0.29	E3
Offspring 2	15	M	152	98	23	100	29	0.23	E3
Offspring 3	18	F	151	45	9	105	37	0.20	E3
Offspring 4	21	F	178	91	32*	103	43	0.35	E3
Family 2									
Sibling 2	49	F	499*	167	196*	244*	59	1.17	E0
Spouse	52	M	233	141	32	156	35	0.23	E3
Offspring 1	21	M	159	85	23	93	43	0.27	E3
Offspring 2	25	M	181	99	24	110	47	0.24	E3
Family 4									
Sibling 4	59	F	237	217	28	156	53	0.24	E3
Family 5									
Proband	60	F	442*	171	160*	193	69	0.94	E0
Spouse	59	M	207	265*	42	112	53	0.16	E4/3
Offspring 1	33	M	240	169	24	171	45	0.14	E4
Offspring 2	38	F	181	111	19	123	39	0.17	E3
Family 7									
Sibling 7	68	F	559*	252*	236*	281*	42	0.94	E0
Offspring 1	43	F	215	42	6	122	87	0.14	E3
Offspring 2	44	M	226	114	30	155	41	0.26	E3
Proband's Mother	87	F	272	239*	43*	176	53	0.18	E3
Paternal Uncle	71	M	323*	234*	66*	211‡	46	0.28	E3
Homozygotes ($n = 4$)	56±11		529±74§	221±62§	243±97§	230±41§	51±16	1.09±0.18§	
Obligate Heterozygotes ($n = 10$)	27±12		191±33	95±36	22±9	123±26	46±16	0.23±0.07	
Normals ($n = 1088$)			189±40	87±43	16±11	123±35	50±14	0.18±0.05	

* Above the 95th percentile of normal based on non-White population, NIH publication 80-1527, p. 52–81, 1980, VLDL C/TG is the VLDL cholesterol to plasma triglyceride ratio. ‡ Above the 90th percentile of normal. § Significantly different ($P < 0.01$) from normal.

saturated ratio (1.0) diet with some caloric restriction resulted in decreases in plasma cholesterol, triglyceride, VLDL cholesterol, and LDL cholesterol of 17, 35, 25, and 36%, with no change in HDL cholesterol. LDL cholesterol includes IDL and LDL cholesterol in these studies. A 3-wk course of ethinyl estradiol 0.1 mg p.o. daily caused increases in plasma triglyceride and HDL cholesterol of 148 and 25% and decreases in total cholesterol, VLDL cholesterol, and LDL cholesterol of 11, 13, and 18%, respectively. Clofibrate administration (1 g p.o., three times daily) resulted in decreases in total cholesterol, triglyceride, and VLDL cholesterol of 7, 34, and 29%, and increases in HDL cholesterol of 66% as compared with values on diet only. The addition of nicotinic acid 1 g p.o., three times daily to this regimen resulted in further decreases in total cholesterol, VLDL cholesterol and LDL cholesterol of 23, 35, and 26%, respectively, with an increase in HDL cholesterol of 10%, and little effect on triglycerides. Despite these decreases, her VLDL cholesterol level was still elevated at 55 mg/dl. Niacin was discontinued because of gastrointestinal side effects. The patient is currently doing well on diet, oral propranolol, clofibrate, and occasional sublingual nitroglycerin. She has two healthy offspring without xanthomas: a son aged 33 yr and a daughter aged 38 yr. Their lipid values are given in Table I. A complete pedigree of the proband's relatives is shown in Fig. 1.

The proband's father died at age 62 yr of a myocardial infarction and coronary artery disease documented at autopsy. He had a history of angina for the previous 12 yr, a history of cigarette smoking, and a long history of xanthomas on his ears, scalp, elbows, and knees. His parents died at the ages of 71 (father) and 75 yr (mother) of suspected coronary artery disease (CAD) and cancer, respectively. None had a history of xanthomas.

The proband's mother was alive and well, except for arthritis, at age 87 yr. Her lipid values are shown in Table I. Her parents died at ages 73 and 80 yr of unknown causes. Two of her siblings died at ages 67 and 71 yr, and one brother was alive and well at 91 yr of age. None of these subjects had a history of xanthomas.

The proband had seven siblings. One brother with no xanthomas and a normal plasma cholesterol expired secondary to kidney failure from glomerulonephritis following unsuccessful kidney transplantation. A sister died at the age of 53 yr due to complications from valvular heart disease. She had no xanthomas, and her seven offspring were healthy with no history of xanthomas. The proband had two other siblings with no history of xanthomas. One sister (sibling 4) was healthy at age 59 yr, as were her three offspring. Her lipid values are shown in Table I. Another sister was healthy at age 65 yr except for a long history of significant hearing loss. Her four offspring were healthy.

The proband had three remaining siblings, all of whom had a history of xanthomas and elevated plasma cholesterol. A younger brother (sibling 1) was first noted to have xanthomas in his 20s, was documented to have type III HLP at age 34 yr, and was subsequently placed on a low saturated fat, low cholesterol diet, as well as clofibrate 500 mg p.o., twice daily.

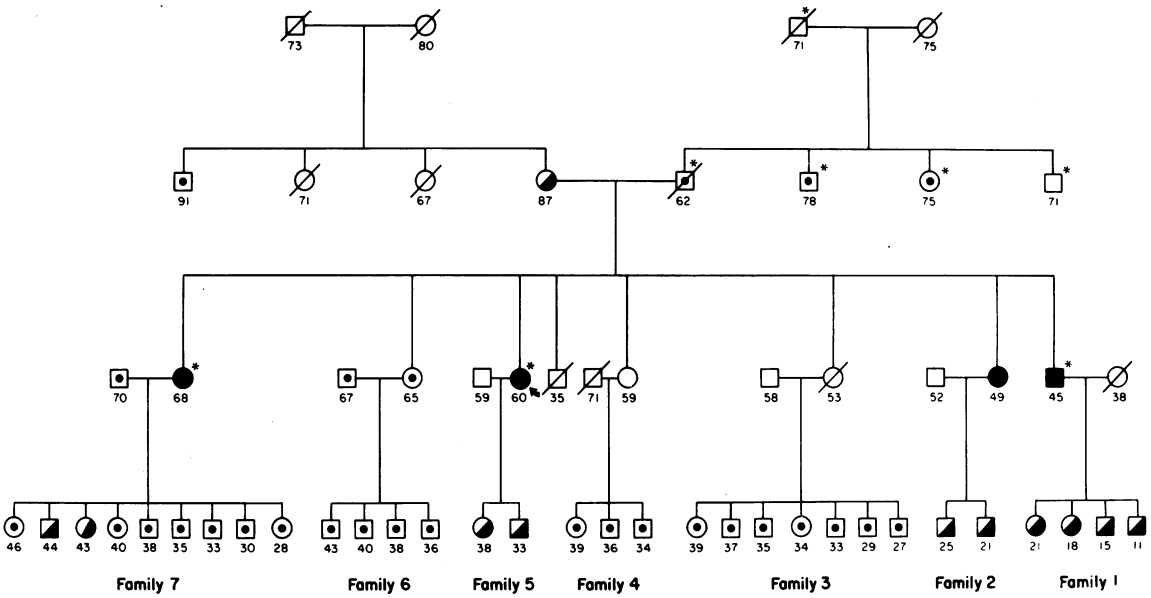

Figure 1. Pedigree of the familial apoE deficiency kindred. ∅, deceased individuals; ⊙, subjects not sampled; •, subjects with documented coronary artery disease or cerebrovascular disease; ●, homozygotes; and ◐ heterozygotes. Criteria for being considered a heterozygote were being an offspring or a parent of a documented homozygote.

The striking xanthomas on his ears, palms, elbows, and knees are shown in Figs. 2 and 3. He also had a history of cigarette smoking and hypertension. At age 46 yr he suffered a mild stroke resulting in temporary right hemiparesis. Better control of his hypertension was achieved, he was advised to quit smoking, and clofibrate was increased to 1 g p.o., twice daily. Off medication his plasma lipid levels are shown in Table I. On medication (clofibrate) his plasma cholesterol, triglyceride, VLDL and LDL cholesterol were decreased by 54, 64, 70, and 57% of corre-

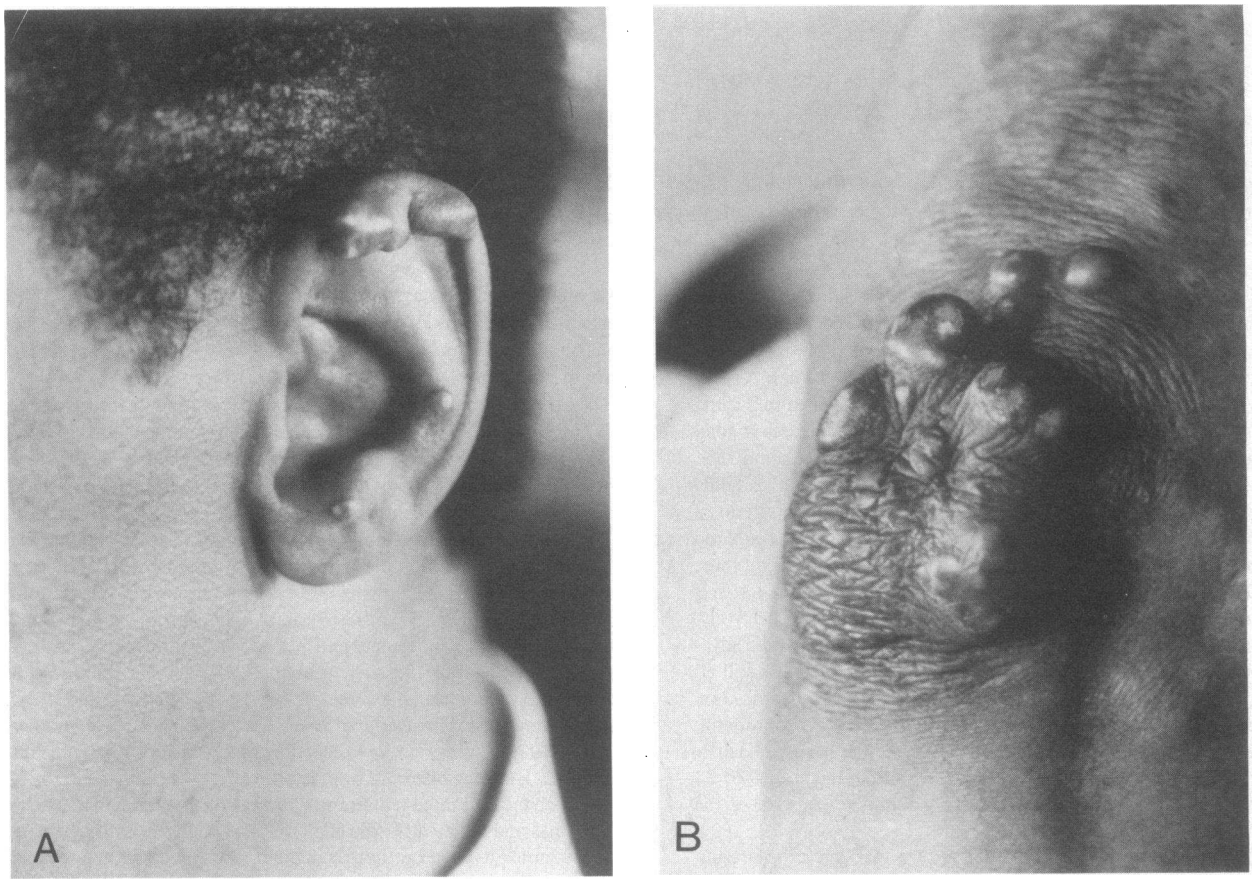

Figure 2. Tubo-eruptive xanthomas on the ears (*A*) and elbows (*B*) in homozygous familial apoE deficiency.

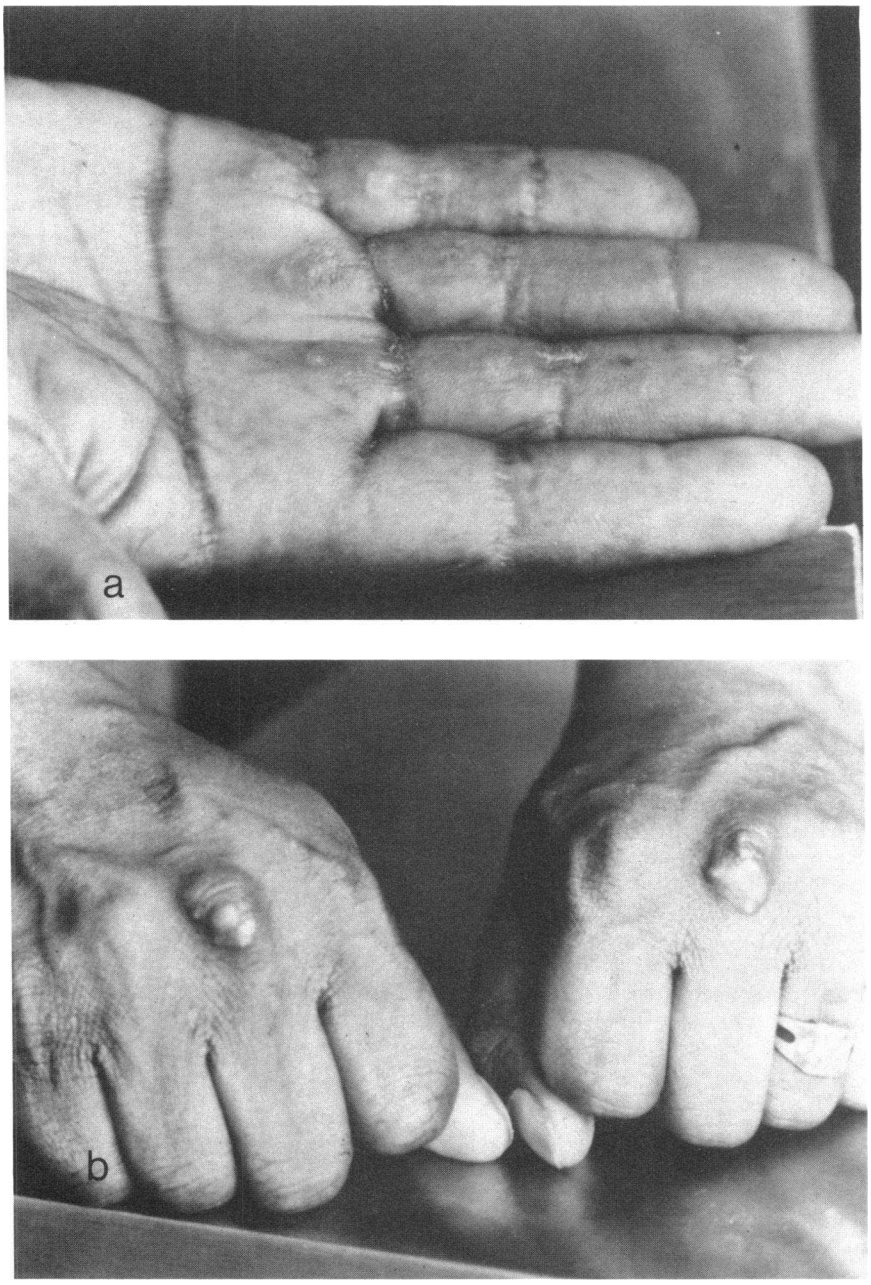

Figure 3. Palmar (*a*) and tubo-eruptive xanthomas (*b*) on the hands in homozygous familial apoE deficiency.

sponding values off medication, while his HDL cholesterol was increased by 109%. The patient was unable to tolerate the addition of regular nicotinic acid because of gastrointestinal distress, and has recently been placed on long-acting nicotinic acid (1 g p.o., twice daily). This therapy resulted in a further decrease in VLDL cholesterol of 27%, with no change in HDL cholesterol. Despite these dramatic reductions, his VLDL cholesterol was still elevated above the 95th percentile of normal at 83 mg/dl. Four of his offspring are alive and well with no xanthomas, and their lipid values are shown in Table I.

One sister (sibling 2) of the proband had a history of tubo-eruptive xanthomas since her 30s. She was noted to be hypercholesterolemic at age 42 yr, and was placed on a low saturated fat, low cholesterol diet, and clofibrate 1 g p.o. twice daily. Her plasma lipid values off medication are shown in Table I. With clofibrate administered at a dosage of 1 g p.o. twice daily she had mean reductions of 27, 50, 61, and 17% in her plasma cholesterol, triglyceride, VLDL cholesterol, and LDL cholesterol, while her HDL cholesterol increased by 59%. Despite these effects her VLDL cholesterol remained elevated at 76 mg/dl. She was unable to tolerate the addition of nicotinic acid because of flushing and gastrointestinal discomfort. Two sons are healthy, and have no xanthomas, and their lipid values were normal (see Table I).

The proband's oldest sister (sibling 7) was 68 yr of age with a history of mild exertional chest pain for the past several years, and a long history of tubo-eruptive xanthomas on her elbows and knees. She was a nonsmoker and was not hypertensive. She was noted to have significant hypercholesterolemia at age 58 yr, and was placed on a cholesterol-lowering diet, which caused a decrease in the size of her xanthomas. She has not as yet been treated with cholesterol-lowering medication. Her lipid values are shown in Table I. Nine offspring were healthy and free of xanthomas.

The parents of the proband and all their siblings were raised in Chatham, Virginia, near the North Carolina border. Their ancestors were involved in agricultural work in this area in the eighteenth and nineteenth centuries, very close to a recently discovered large uranium deposit (the largest known deposit in the eastern United States). Two of the wells in the immediate area where the parents of the proband lived have recently

been closed because of high radioactivity levels in the water. No consanguinity could be documented. Consanguinity is a possibility, however, since paternal and maternal grandparents lived in the same area, and were part of a small rural Black community.

Genetic studies. All living relatives as listed on the pedigree (Fig. 1) were contacted by mail and telephone, and information about their health status and physical findings obtained from them as well as their local physicians. 14 kindred members including three homozygotes and the proband's mother were personally evaluated by the first author, and blood samples obtained in 0.1% EDTA in the fasting state (12 h). On six additional kindred members, including sibling 7 of the proband, blood samples were obtained in identical fashion and shipped to our laboratory on ice by overnight air express. All blood samples were obtained while subjects had been off lipid lowering medication for at least 3 wk and while on an ad lib. diet, except for homozygous siblings 1, 2, and 7, whose blood samples were examined while on an outpatient low saturated fat, low cholesterol diet. These samples were used to generate data presented in Table I.

Lipoprotein analysis. Plasma was subjected to ultracentrifugation at $d = 1.006$ g/ml for 18 h at 39,000 rpm in an (L265b, Beckman Instruments, Inc., Fullerton, CA) ultracentrifuge. Cholesterol and triglyceride in plasma, the 1.006-g/ml infranate, and the dextran magnesium sulfate supernate fraction (HDL) were determined by enzymatic methods as previously described (15). VLDL cholesterol and LDL cholesterol were calculated by difference from these data by standard Lipid Research Clinics procedure (see Table I) (16). Therefore LDL cholesterol values in Table I represent the sum of IDL and LDL cholesterol. Plasma and the 1.006-g/ml supranatant and infranatant fractions were subjected to lipoprotein paper electrophoresis (16). Plasma from the proband and her two offspring was also subjected to 0.5% agarose electrophoresis as previously described (4). Plasma samples obtained in the fasting state from three homozygotes (proband, siblings 1 and 2) and six normal control subjects were also subjected to sequential ultracentrifugation in order to isolate VLDL ($d < 1.006$ g/ml), IDL (d, 1.006–1.019 g/ml), LDL (d, 1.019–1.063 g/ml), HDL_{2b} (1.063–1.10 g/ml), and HDL_{2a+3} (d, 1.10–1.21 g/ml) as previously described (17). Protein, phospholipid, cholesterol, and triglyceride concentrations in lipoprotein fractions were determined in triplicate as previously described (see Table II) (17). Free cholesterol in plasma was determined by enzymatic methods (17). Analytical ultracentrifugation of plasma from the proband and her two offspring was performed, and computer-derived patterns were obtained by procedures that corrected for the concentration dependence of flotation rate and the Johnston-Ogston effect (18).

Electron microscopy. Lipoprotein fractions from the proband were dialyzed overnight against 0.13 M ammonium acetate buffer, pH 7.4 containing 0.35 mM EDTA and 0.125 mM merthiolate. Samples were negatively stained with 2% sodium phosphotungstate, pH 7.4, and immediately examined in the JEM 100C electron microscope (JEOL, Inc., Tokyo, Japan). Sizes of lipoprotein particles were obtained from 200 free standing particles per fraction according to procedures previously described (19).

Polyacrylamide gradient gel electrophoresis. Precast 2–16% polyacrylamide agarose slab gels (PAA 2/16, Pharmacia Fine Chemicals, Piscataway, NJ) were used to estimate size and heterogeneity of VLDL, IDL, and LDL fractions from the proband. The method described by Krauss and Burke (20) was employed for running and staining the gels, and carboxylated latex beads (Dow Chemical Co., Indianapolis, IN), thyroglobulin and apoferritin were used as standards to estimate particle radius. HDL fractions (HDL_{2b}, d 1.063–1.10 g/ml; and $HDL_{2a} + 3$, d 1.10–1.21 g/ml) were analyzed on 4–30% polyacrylamide precast gels (PAA 4/30, Pharmacia Fine Chemicals) according to the procedure of Blanche et al. (21). Reference proteins used to determine particle radius consisted of thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin. All gels were stained with Coomassie G-250 and scanned with a densitometer (model RFT, Transidyne Corp, Ann Arbor, MI) at a wavelength of 596 nm.

Apolipoprotein analysis. Plasma apolipoprotein A-I, A-II, B and C-II concentrations as well as apoB values in the 1.006-g/ml infranate were measured by radial immunodiffusion as previously described (17). Plasma apoE levels were determined by radioimmunoassay as previously described (22). Group 1 normal subjects (mean age 21±2 yr, 25 males, 25 females) served as controls for subjects under 30 yr of age, while group 2 normal individuals (mean age 37±5 yr, 20 males, 11 females) served as controls for subjects 30 yr of age or older. Plasma and VLDL total apoB and apoB-100 in fasting plasma and VLDL obtained from three homozygotes and five normal subjects was also determined by enzyme

Table II. Plasma Lipoprotein Composition

	Protein	Phospholipid	Cholesterol	Triglyceride
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*
VLDL				
Homozygotes	31.8±5.5 (9.3)*	60.5±11.7 (17.7)*	172±55 (50.4)*	77±25 (22.5)*
Normals	15.3±1.3 (13.6)	34.8±6.1 (31.0)	11±2 (9.8)	51±9 (45.5)
IDL				
Homozygotes	22.5±4.9 (7.9)*	32.6±13.1 (11.4)*	161±35 (56.4)*	69±24 (24.2)*
Normals	4.2±0.7 (11.9)	16.2±2.3 (45.8)	3±1 (8.5)	12±4 (33.9)
LDL				
Homozygotes	79.2±12.1 (27.8)	63.0±5.1 (22.1)	93±5 (32.6)	31±11 (10.9)
Normals	78.5±2.7 (32.5)	60.0±6.0 (24.8)	81±6 (33.5)	22±1 (9.1)
HDL_{2b}				
Homozygotes	26.2±7.1 (44.2)	13.1±3.2 (22.1)	14±4 (23.6)	6±2 (10.1)
Normals	22.5±6.6 (37.7)	15.2±1.5 (25.5)	14±2 (23.5)	8±1 (13.4)
HDL_{2a+3}				
Homozygotes	87.6±7.3 (57.4)	29.0±3.7 (19.0)	28±5 (18.3)	8±2 (5.2)
Normals	83.2±7.3 (52.2)	40.2±1.8 (25.2)	26±2 (16.3)	10±2 (6.3)

Mean values±SD; numbers in parentheses represent percentage of total lipoprotein by weight; * Significantly different ($P < 0.01$) from normal by chi square analysis; VLDL ($d < 1.006$ g/ml), IDL (d, 1.006–1.019 g/ml), LDL (d, 1.019–1.063 g/ml), HDL_{2b} (d, 1.063–1.10 g/ml), and HDL_{2a+3} (d, 1.10–1.21 g/ml); analysis performed on six normolipidemic control subjects and three homozygotes (proband, siblings 1 and 2), cholesterol represents the sum of free and esterified cholesterol.

linked immunoassay utilizing polyclonal and monoclonal antibodies as previously described (23). In addition, plasma samples on the proband were shipped on dry ice via overnight air freight to the laboratory of Dr. Conrad Blum, Department of Medicine, Columbia Presbyterian Hospital, New York, NY, for the determination of apoE by radioimmunoassay, and to the laboratory of Dr. Petar Alaupovic, Oklahoma Medical Research Foundation, Oklahoma City, OK, for the measurement of plasma apolipoproteins C-I, C-III, D, E, and F by electroimmunoassay as previously described (24, 25). Delipidated VLDL on all subjects, and delipidated lipoprotein fractions on three homozygotes (proband, siblings 1 and 2) were subjected to isoelectric focusing (pH 4–6.5, 7.5% acrylamide) as well as 15 and 3.5% polyacrylamide sodium dodecyl sulfate gel electrophoresis, as previously described (12, 13, 26, 27). Delipidation of lipoproteins was carried out by chloroform/methanol extraction (vol/vol 2:1) (26).

Metabolic studies. The proband and 11 normal subjects (6 males, 5 females) aged 19–25 yr, were hospitalized in the Clinical Center, National Institutes of Health. Data on 9 of the normal subjects have been previously reported (28). All subjects had normal fasting glucose levels, were euthyroid, and had normal liver and renal function tests. None of the subjects including the proband was on any medication during the study. All normal subjects had the apoE3/3 phenotype. Study subjects were placed on a defined iso-weight diet containing 42% of calories as carbohydrate, 42% as fat, 16% as protein, 200 mg cholesterol per 1,000 kcal, and a polyunsaturated/saturated fat ratio of 0.1:0.3 for ~1 wk before study (28). 3 d prior to injection of the tracer, the diet was changed to a liquid formula of the same nutrient composition and was given every 6 h. All subjects received potassium iodide (1,200 mg/d) and ferrous gluconate (900 mg/d) in divided doses. Nine normal subjects were injected intravenously with 25 μCi of ^{131}I or 25 μCi of ^{125}I-radiolabeled apoE3 ~5½ h after their previous meal (28).

ApoE3 was isolated, radioiodinated, and added to lipoproteins as previously described (28). ApoE was iodinated by the iodine monochloride method with an efficiency of 35–50% with ~0.5 mol of iodine per mol of protein (28). Radioiodinated apoE was incubated (30 min, 37°C) with plasma (75 ml) and plasma lipoproteins ($d < 1.21$ g/ml) were isolated. Radiolabeled apoE associated with lipoproteins was used as the tracer (28). The proband and two normal subjects received 25 μCi of ^{131}I-apoE3 associated with plasma lipoproteins isolated from the proband in the fasting state. One of these normal subjects simultaneously received 25 μCi of ^{125}I-apoE3 associated with normal plasma lipoproteins (28).

The proband and one normal subject also received 50 μCi of ^{125}I-VLDL isolated from the proband. VLDL was isolated by ultracentrifugation from the proband's plasma obtained by plasmapheresis (160 ml) using a Beckman 60 Ti rotor at 59,000 rpm for 15 h. A second ultracentrifugation step was carried out following overlayering with 1.006 g/ml sterile density solution containing 0.01% EDTA, 150 mM NaCl, 0.1 M Tris HCl, (pH 7.4). Isolated VLDL was radioiodinated as previously described (29). The amount of ICl added was calculated so that ~0.5 mol of iodine were bound per mole of VLDL protein, assuming a molecular weight of 250,000 for VLDL protein (29). The efficiency of iodination was 16%, and 12% of the covalently bound radioactivity was present on lipid. All injected tracers were sterilized by passage through a 0.22-μm filter (Millipore Corp., Bedford, MA), were pyrogen free, and were injected in sterile 1% human albumin solution.

During the course of the metabolic studies, blood samples (20 cm^3) were obtained in 0.1% EDTA at 10 min, 6, 12, 18, 24, 36, and 48 h, and daily prior to breakfast through day 7. In subjects receiving ^{125}I-VLDL, blood samples were also obtained at 2 min, as well as 1, 3, and 9 h after injection. Plasma was separated (2,500 rpm, 30 min, 4°C), sodium azide and aprotinin added to final concentrations of 0.05% and 200 kallikrein inhibition units (KIU)/ml, respectively, and aliquots of plasma were frozen at −20°C for apoE quantitation by radioimmunoassay (22). Plasma lipoproteins were isolated from all samples by ultracentrifugation as previously described (19). From the proband and the normal subject who received ^{125}I-VLDL, VLDL ($d < 1.006$ g/ml), IDL (d, 1.006–1.019 g/ml), and LDL (d, 1.019–1.063 g/ml) were delipidated with chloroform/methanol (2:1, vol/vol). The amount of radioactivity in the lipid fraction was quantitated. Aliquots of VLDL, IDL, and LDL protein (100 μg) from all time points were subjected to 3.5% polyacrylamide sodium dodecyl sulfate gel electrophoresis as well as 15% polyacrylamide gel electrophoresis (PAGE) as previously described (12, 17, 26). Radioactivity in plasma, lipoprotein fractions, lipid, and isolated gel slices was quantitated in a gamma counter (Autogamma 5260, Packard Instrument Co., Inc., Downers Grove, PA). Specific protein bands examined were apoB-100 and apoB-48. Disappearance of ^{131}I-radioactivity was also monitored with a whole body counter (30).

All gel segments were counted, and apoB-100 and apoB-48 radioactivity was determined after subtracting counts obtained from control gel slices of identical length containing no visible protein band. ApoB-100 and apoB-48 radioactivity from all time points utilized in all fractions was at least fivefold higher than the amount of radioactivity in control slices. Recovery of radioactivity applied to 15% PAGE was 93.1±3.2%, and 44.2±4.1% of initial VLDL radioactivity was in the apoB fraction. ApoB-100 and apoB-48 specific radioactivity was determined as a fraction of initial total apoB radioactivity (based on 3.5% gels). Total apoB radioactivity was determined as a fraction of initial apolipoprotein radioactivity within VLDL, IDL, or LDL (based on 15% PAGE). Radioactivity within each lipoprotein fraction isolated from plasma was measured by counts in each fraction following isolation and after subtracting off the counts associated with lipid labeling.

In order to further assess the metabolism of apoB-100 and apoB-48, the kinetics of ^{125}I-chylomicrons were investigated in two normolipidemic subjects undergoing thoracic duct drainage for purposes of immunosuppression following successful kidney transplantation. These subjects were studied at Walter Reed Army Medical Center, Washington, DC. Two female subjects, ages 37 and 45 yr, were studied while on an ad lib. diet of normal composition. Both subjects had normal thyroid and liver function, and their mean creatinine was only slightly above normal at 1.6 mg/dl. Lymph drainage was accomplished via a surgically implanted thoracic duct catheter, and lymph was replaced following lymphocyte removal at approximately the same rate that it was being drained (~4 liter/d). Chylomicrons were isolated from lymph using an SW 27 Beckman rotor by ultracentrifugation at 27,000 rpm for 30 min at 28°C, with three successive spins to minimize contamination with other lipoproteins. Chylomicrons were labeled by the iodine monochloride method as previously described (31). Efficiency of iodination was 25% and lipid labeling was 15%. Each subject received 50 μCi of autologous ^{125}I-chylomicrons and other aspects of the study were carried in identical fashion to the ^{125}I-VLDL studies previously described except that blood samples were only obtained up to 48 h after injection.

All patients were studied under approved Human Investigation Review Committee protocols at the National Institutes of Health and Walter Reed Army Medical Center. Informed consent was obtained from all subjects.

Kinetic analysis. Plasma, whole body, and specific apolipoprotein radioactivity decay curves were fit with three exponentials with the SAAM simulator program on a VAX 11/780 Digital Computer (Digital Corp., Maynard, MA). Residence times were calculated from the areas under the decay curves (30). Residence time is the reciprocal of the fractional catabolic rate. Under steady state conditions: synthesis rate (mg/kg d) = [(plasma concentration, mg/dl) (plasma volume, dl)] ÷ [(residence time, days) (body wt, kg)]. ApoB-100 and apoB-48 radioactivity decay was determined as a percentage of initial radioactivity. ApoE plasma kinetic parameters were determined as previously described (28). The plasma volume for all study subjects was determined by isotope dilution at the first time point.

Results

Lipoprotein analysis. Data on age, gender, plasma lipid, and lipoprotein cholesterol concentrations, and apoE phenotype are given in Table I. Obligate heterozygotes (offspring of homozygotes) had mean plasma lipid and lipoprotein cholesterol values within the 5th–95th percentile of the normal non-White pop-

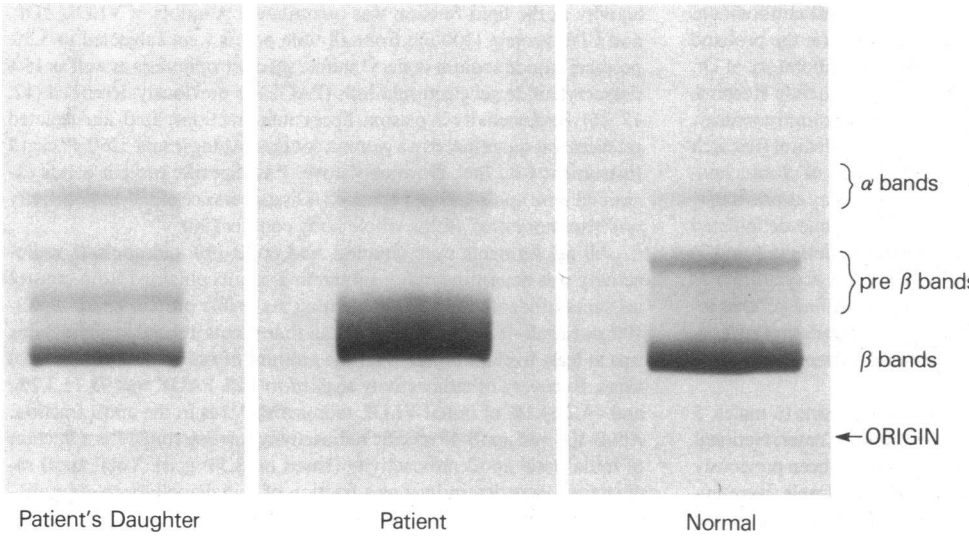

Figure 4. Plasma lipoprotein electrophoresis in 0.5% agarose demonstrating a broadly migrating band between the prebeta and beta lipoproteins in the homozygous proband's plasma (labeled patient), and the presence of two prebeta bands in the plasma of the proband's daughter (labeled patient's daughter). A normal pattern is shown at right.

ulation except for two offspring of sibling 1 of the proband, who had slightly elevated VLDL cholesterol levels (32). In addition, the proband's mother and a paternal uncle both had hyperlipidemia. These subjects are probably also heterozygotes. The four homozygotes sampled had significant hyperlipidemia associated with elevations in both VLDL and LDL cholesterol (includes both IDL and LDL), and a marked elevation in the VLDL cholesterol/plasma triglyceride ratio. Lipoprotein electrophoresis of plasma in homozygotes was consistent with type III hyperlipoproteinemia with a broad beta band, and two obligate heterozygotes tested had an increased slow prebeta lipoprotein band (Fig. 4). This band is found in 30% of normal subjects. All subjects had standard paper lipoprotein electrophoresis, and a beta migrating VLDL band was only observed in the four homozygotes. Analytical ultracentrifugation of the proband's plasma indicated a significant increase in VLDL and IDL and no clear LDL peak (see Fig. 5). Lipoprotein compositional analysis in three homozygotes demonstrated a significant increase in VLDL and IDL protein, phospholipid, cholesterol, and triglyceride concentrations (see Table II). No increases in LDL (*d*, 1.019–1.063 g/ml) constituents were noted. The percentage of plasma cholesterol in the unesterified form was normal (28±2%) in three homozygotes.

Electron microscopy and gradient gel electrophoresis. Particle size distributions of plasma lipoproteins obtained from the proband in the fasting state were analyzed by nondenaturing gradient gel electrophoresis and electron microscopy as shown in Fig. 6. Electron microscopy revealed no abnormal morphology and mean particle diameters (nm±SD) based on electron microscopy were: VLDL, 36.5±11.5; IDL, 31.0±3.9; LDL, 26.9±4.2; HDL_{2b}, 10.5±1.3; and HDL_{2a+3}, 8.3±1.7. VLDL particles were extremely heterogeneous in size as indicated by the large standard deviation; this heterogeneity was confirmed by scanning of gradient gels that demonstrated the presence of at least four components. The shoulder at 29.7 nm, however, probably represents overlap with IDL. Scans of the IDL gradient gel pattern demonstrated two peaks at 29.9 and 29.1 nm. Gradient gel analysis of LDL was also consistent with significant heterogeneity in this lipoprotein density fraction with at least four peaks in evidence (see Fig. 6). The LDL components with diameters of 28.6 and 27.8 nm probably represent overlap with IDL. Gradient gel analysis of HDL subfractions were consistent with electron microscopy (data not shown) indicating normal sized HDL particles in the HDL_{2b} and HDL_{2a+3} density fractions.

Apolipoprotein analysis. Apolipoprotein analyses of lipoprotein fractions by gel electrophoresis as shown in Figs. 7 and 8 were carried in normal subjects (n = 5), two obligate heterozygotes, and three homozygotes. These data indicate lack of apoE and accumulation of apoB-48 and apoA-IV in the VLDL, IDL, and LDL density fractions of subjects with homozygous apoE deficiency. Consistent results were obtained in all three homozygotes tested. These apolipoproteins were not observed in lipoproteins obtained from normal subject or heterozygotes in the fasting state. In the postprandial state apoB-48 and apoA-IV were observed in normal subjects in the 1.006-g/ml supranatant fraction only. The apoB-48 band had an estimated molecular weight that was ~50% of the major protein component of plasma

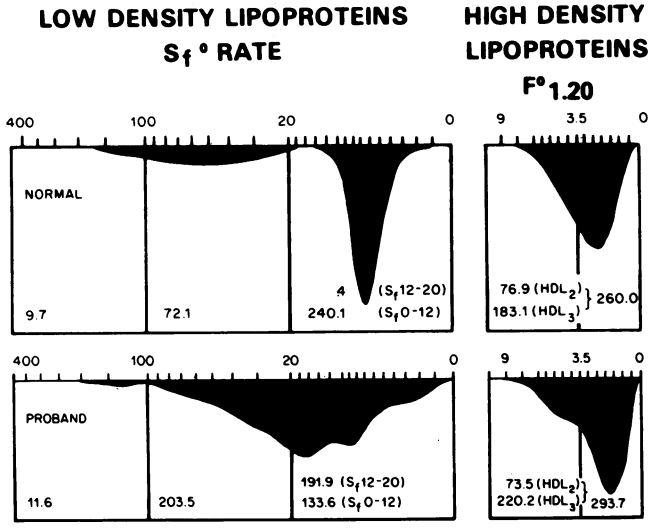

Figure 5. Analytical ultracentrifugation lipoprotein pattern in plasma from a normal subject (*top*) and the proband (*bottom*) demonstrating accumulation of lipoproteins in the VLDL and IDL region, and lack of a normal LDL peak in the proband's plasma.

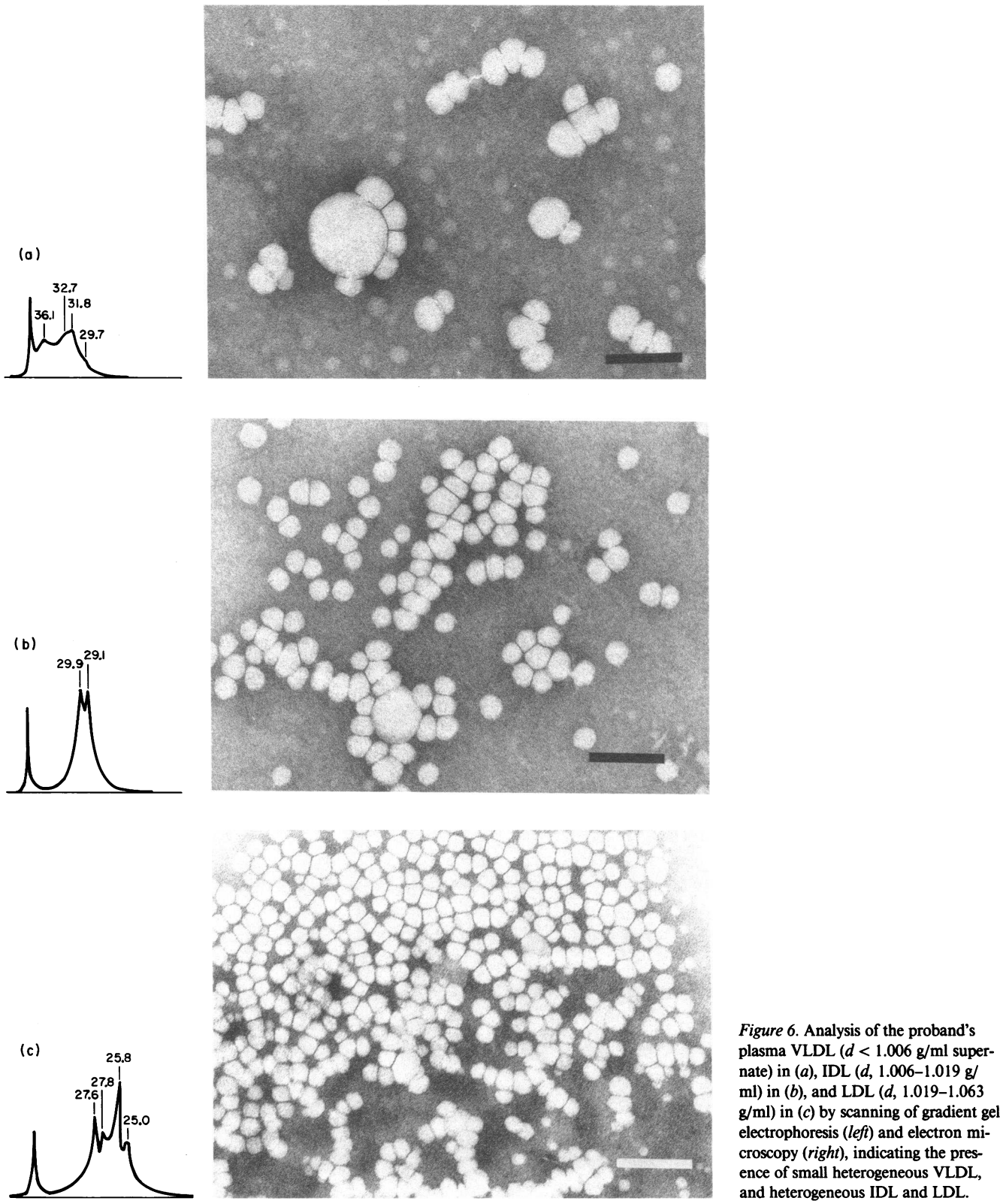

Figure 6. Analysis of the proband's plasma VLDL ($d < 1.006$ g/ml supernate) in (*a*), IDL (*d*, 1.006–1.019 g/ml) in (*b*), and LDL (*d*, 1.019–1.063 g/ml) in (*c*) by scanning of gradient gel electrophoresis (*left*) and electron microscopy (*right*), indicating the presence of small heterogeneous VLDL, and heterogeneous IDL and LDL.

LDL, and had identical mobility to the major apoB form in human thoracic lymph chylomicrons. Based on scanning densitometry of VLDL apolipoproteins run on 3.5% gels for two homozygotes, the mean apoB-100: apoB-48 ratios were 4.7 and 5.1, respectively. In five normal subjects, this ratio was in excess of 90. Based on a sensitive enzyme-linked immunoassay for total apoB and apoB-100 in normal subjects over 98% of apoB in VLDL and plasma (fasting) was in the form of apoB-100, while

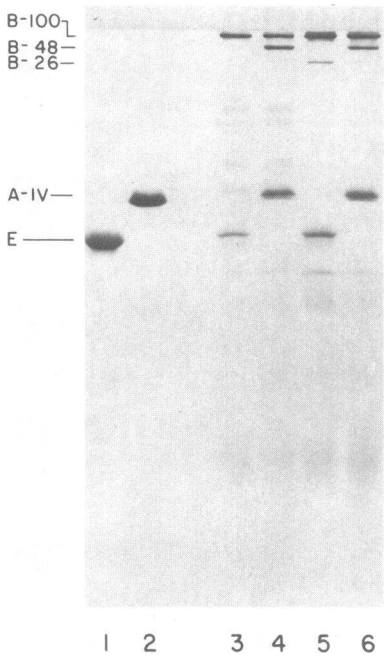

Figure 7. PAGE (15%) of delipidated VLDL ($d < 1.006$ g/ml) from a normal subject (lane *3*) and the proband (lane *4*) and delipidated plasma LDL (*d*, 1.019–1.063 g/ml) from a normal subject (lane *5*) and the proband (lane *6*) demonstrating the accumulation of apoB-48 and apoA-IV and lack of apoE in the proband's VLDL and LDL. Purified apoE (lane *1*) and apoA-IV (lane *2*) are shown. 20 μg of protein were loaded onto each lane of the slab gel.

in two homozygotes these percentages were 82.5 and 91.1%, respectively.

Plasma apolipoprotein concentrations in homozygotes, obligate heterozygotes, and normal subjects are shown in Table III. Mean plasma apo A-I, A-II and C-II concentrations were significantly higher in homozygotes than in normals. Normal ranges for apoA-I and apoA-II plasma concentrations in Black and White subjects from Evans County studies are also given as derived from published data (33). ApoB concentrations in the 1.006-g/ml supernatant fraction were also significantly higher in this group than in normals. ApoE was undetectable in the plasma of homozygotes by immunoblotting or immunoelectrophoresis but trace amounts were detectable by radioimmunoassay as shown in Table III. Mean apoE concentrations in homozygotes were 0.3% of those observed in normal subjects. Parallelism of values for apoE by dilution could not be shown because these values were very close to the detection limits of the assay. However, values obtained were consistently significantly above background. Obligate heterozygotes had normal plasma apolipoprotein concentrations except that their mean apoE levels were 42.1% of those observed in normal subjects ($P < 0.01$, t test analysis).

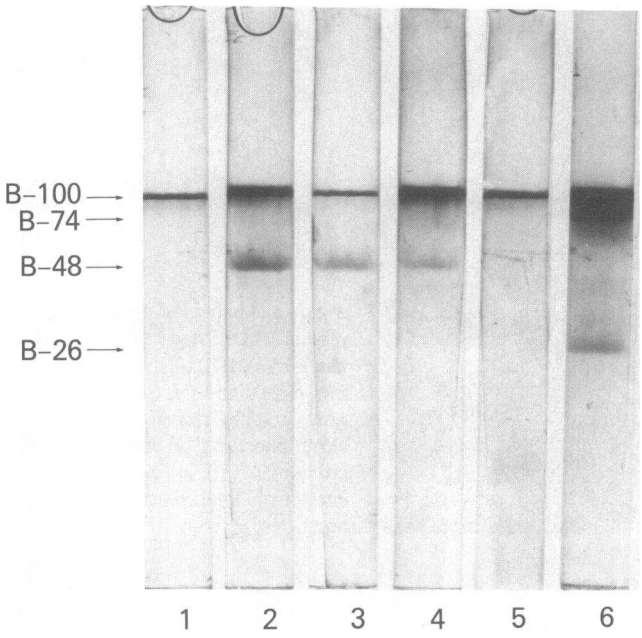

Figure 8. PAGE (3.5%) of VLDL ($d < 1.006$ g/ml), IDL (*d*, 1.006–1.019 g/ml) and LDL (*d*, 1.019–1.063 g/ml) for a normal subject (lanes *1*, *5*, and *6*) and for the proband (lanes *2–4*) demonstrating apoB-48 accumulation in the lipoproteins of the proband.

Apolipoprotein kinetic studies. The results of kinetic studies are shown in Table IV, and Figs. 9 and 10. In three normolipidemic subjects the mean residence time (±SD) of VLDL apoB-100 (0.12±0.02 d or 2.9 h) was significantly greater than that observed for VLDL apoB-48 (0.04±0.01 d or 1.0 h) whether these apolipoproteins were labeled and injected as VLDL isolated from the apoE-deficient proband or as thoracic duct lymph chylomicrons. In contrast to VLDL apoB-100, almost no VLDL apoB-48 radioactivity was transferred to LDL (1.3% of total apoB-48 radioactivity, see Fig. 9). In the apoE-deficient proband, the VLDL apoB-100 residence time was 7.8 times greater than that observed in normals, and the apoB-48 residence time was 44 times greater than normal (see Table IV, Fig. 9). Similar to normals, only a small fraction of VLDL apoB-48 radioactivity was transferred to LDL in the apoE-deficient proband but the apoB-48 that was transferred, was catabolized very slowly from LDL (see Fig. 9). While apoB-100 decay was slower than normal in the VLDL fraction, it appeared to be similar to normal in the LDL fraction in the proband (see Table IV, Fig. 9). The calculated VLDL apoB-100 synthesis rate for the proband was normal at 9.49 mg/kg per d (29).

ApoE kinetics were also assessed in normal subjects and the proband (see Table IV, Fig. 10). Radioiodinated apoE3 injected on the proband's lipoproteins decayed significantly slower (residence time was fivefold greater) in the proband than was observed in normal subjects ($n = 2$) receiving the identical tracer. It should also be noted that the apoE3 tracer injected into a normal subject on the proband's 1.21-g/ml supernatant fraction had a residence time that was 55% of that observed for apoE3 injected on normal 1.21-g/ml supernatant fraction in the same subject. The calculated apoE synthesis rate in the proband was only 0.15% of that observed in normal females.

Discussion

We have previously reported data on plasma lipid and lipoprotein cholesterol analysis, SDS PAGE, and Ouchterlony immunodiffusion for the proband, two offspring, and two affected siblings in this unique kindred with familial apolipoprotein E deficiency (12). In the present report we extend these observations to include a complete clinical description and biochemical characterization of the entire kindred, as well as apolipoprotein kinetic studies. The genetic pattern in this kindred is most consistent with an autosomal recessive model of inheritance in which only ho-

Table III. Apolipoprotein Concentrations (mg/dl)

Subjects	ApoA-I	ApoA-II	ApoC-II	ApoB	ApoB in 1.006 T*	ApoB in 1.006 B*	ApoE
Homozygotes							
Proband	176‡	43‡	6.1‡	111	27‡	84	0.016‡
Sibling 1	125	42‡	8.9‡	127§	35‡	92	0.014‡
Sibling 2	188‡	50‡	4.8§	116	29‡	87	0.019‡
Sibling 7	147§	39‡	5.7‡	135§	35‡	100	0.013‡
Mean±SD ($n = 4$)	159±28‖	44±5‖	6.4±1.8‖	122±11	32±3‖	91±7	0.016±0.003‖
Obligate heterozygotes ($n = 8$)	121±18	32±3§	4.1±1.2§	104±12	12±2	92±6	2.4±1.4‡‖
Normal (group 1) ($n = 50$)	117±17	27±4	3.1±1.1	82±25	7±3	94±23	5.7±1.4
Normal (group 2) ($n = 31$)	137±20	32±4	4.1±1.6	96±29	8±2	88±25	
Normal (group 3)							
Black males ($n = 68$)	122±33	39±16					
White males ($n = 82$)	110±35	38±14					
Black females ($n = 73$)	135±38	38±11					
White females ($n = 95$)	127±35	37±11					

* 1.006 T and 1.006 B represent 1.006 g/ml supernate and infranate, respectively. § Value > 1 SD above or below the normal mean. ‡ Value > 2 SD above or below the normal mean. ‖ Significantly different ($P < 0.01$) from normal values, group 1 normal subjects had mean (±SD) age of 21±2 yr, for group 2 normal subjects this value was 37±5 yr, group 3 normal ranges derived from reference 33.

mozygotes develop tubo-eruptive xanthomas and type III HLP. ApoE levels in plasma obtained from heterozygotes are about 50% of normal; therefore the specific biochemical abnormality is expressed in a codominant fashion. It is most likely that the proband's father was a homozygote since he had xanthomas, and that the proband's mother was a heterozygote. Whether apoE deficiency alone was sufficient to cause type III hyperlipoproteinemia in homozygotes or whether an additional genetic factor is present in this kindred that is predisposed to hyperlipidemia is not certain. The moderate elevations of VLDL cholesterol observed in the proband's mother, a paternal uncle, and in 2 of 10 obligate heterozygous offspring of homozygotes may have been due to the presence of another genetic lipoprotein disorder in this kindred or to heterozygous familial apolipoprotein E deficiency. However, the observation that all four homozygotes had severe type III hyperlipoproteinemia with a VLDL cholesterol/plasma triglyceride ratio over 0.90, never observed in other hyperlipidemic subjects to our knowledge, supports the concept that familial apolipoprotein E deficiency alone is responsible for the lipoprotein abnormalities observed in homozygotes and obligate heterozygotes (34–36).

About 50% of the offspring of the proband's parents were homozygotes, and no homozygotes have been found among the offspring of the proband or any of her homozygous siblings. The xanthomas that the proband's brother had are unusual in their location on the ears as well as in other areas. The proband's father apparently had similar xanthomas. Xanthomas in this location have not been reported in patients with other forms of

*Table IV. Metabolic Data**

Subject	Height	Weight	Plasma volume	Plasma Apo E	Synthesis rate	Residence time		
						ApoE	VLDL apoB-100	VLDL apoB-48
	cm	*kg*	*cm³*	*mg/dl*	*mg/kg per d*	*d*	*d*	*d*
Proband§	161	67.2±0.2	2413	0.016‡	0.004‡	1.50‡	0.93	1.76
Normal subjects								
Female§ 1	155	57.7±0.1	2213	4.3		0.30‡	0.09	0.03
Female‖ 2	152	53.1±0.1	2010	—	—	—	0.13	0.05
Female‖ 3	153	55.2±0.1	2141	—	—	—	0.14	0.04
Male¶ A	182	76.5±0.1	3971	3.2		0.29‡	—	—
B	—	—	—	—	3.19	0.52		
Normal females ($n = 6$)**	167±4	56.3±1.1	2592±269	4.5±0.5	2.60±0.80	0.83±0.16	—	—
Normal males ($n = 6$)**	183±2	80.1±7.4	4055±240	5.1±1.5	4.20±1.73	0.63±0.15	—	—

* Values given as mean±SD. ‡ 2 SD above or below the normal mean. § The proband received autologous ^{125}I VLDL and ^{131}I-apoE3 on autologous plasma, female 1 received the same tracers as the proband. ‖ Females 2 and 3 received autologous ^{125}I-lymph chylomicrons. ¶ One normal male received radiolabeled apoE3 on the proband's 1.21-g/ml supernate (study *A*) and also received radiolabeled apoE on his own 1.21-g/ml supernate (study *B*). ** All other normal subjects received radiolabeled apoE3 on normal 1.21 g/ml supernate.

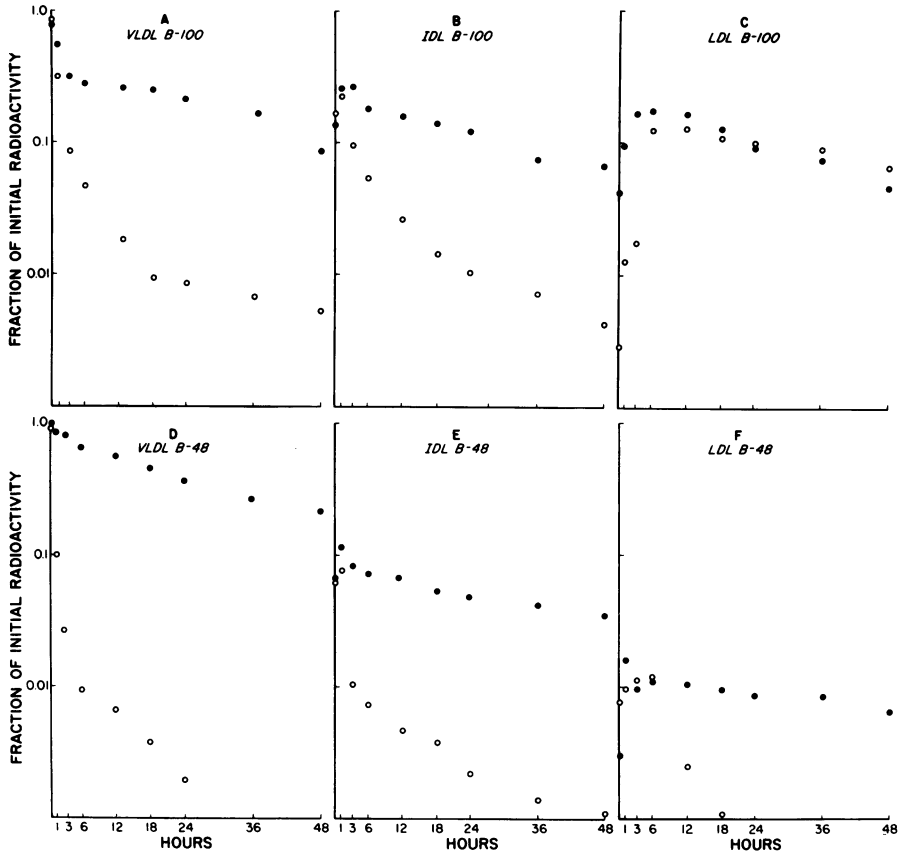

Figure 9. VLDL, IDL, and LDL apoB-100 (*top*) and apoB-48 (*bottom*) radioactivity decay in a normal subject (○) and the proband (●) demonstrating delayed catabolism of VLDL, IDL, and LDL apoB-48 and VLDL and IDL apoB-100 in the proband. ^{125}I-VLDL (isolated from the proband) was utilized for these experiments.

type III HLP (1–3). Whether the presence of a large uranium deposit in the Chatham, Virginia area could have contributed to this mutation is not clear. Radiation exposure has clearly been shown to increase the rate of mutations in a variety of organisms (37). While no consanguinity was documented, it is quite possible that the parents of the proband were related since all their forefathers had been in the Chatham area in the 1800s as part of a small community.

Premature CAD and stroke events were evident among homozygotes, but were not strikingly premature. The proband developed angina prior to age 60 yr, sustained a small subendocardial myocardial infarction, and had single-vessel coronary disease that was treated with angioplasty. Sibling 7 was 68 yr of age with a long history of mild angina, but no history of myocardial infarction, and another homozygous sibling (No. 2) was asymptomatic at age 49 yr. The affected males in the kindred

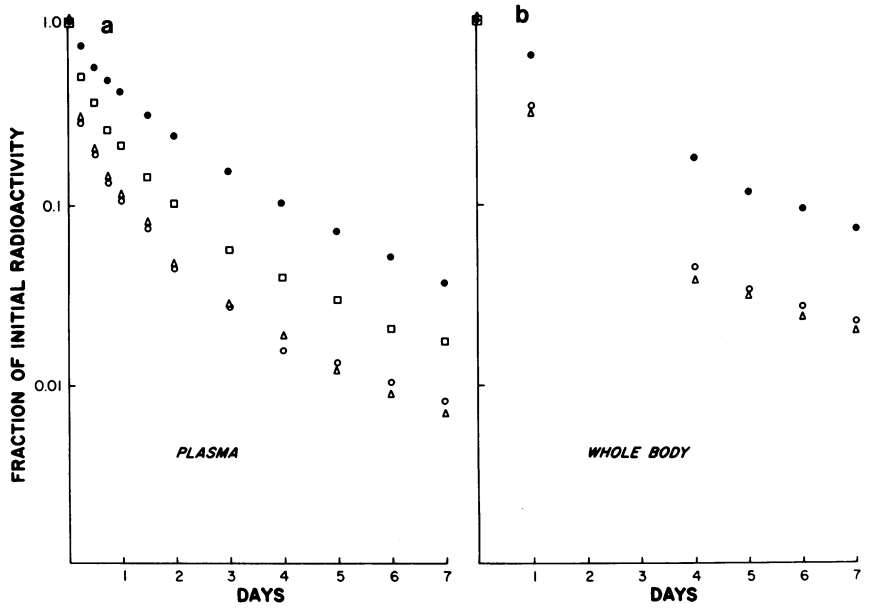

Figure 10. Plasma (*a*) and whole body (*b*) apoE kinetics in the proband (●) and two normal subjects (△, ○). Radiolabeled ^{131}I-apoE3 incubated with the proband's plasma and reisolated in 1.21 g/ml supernate was utilized. One normal subject (□) simultaneously received ^{125}I-apoE3 incubated with autologous plasma with subsequent isolation of the 1.21 g/ml supernate for injection. ApoE was catabolized at a slower fractional catabolic rate in the proband than in normal subjects.

have not been as fortunate; the proband's father (probable homozygote) developed angina in his 50s, and died of a documented coronary occlusion at age 62 yr, and the homozygous brother had a mild stroke in his 40s. Other risk factors in addition to hyperlipidemia may have played a role in the pathogenesis of the premature atherosclerosis observed in these subjects. In addition, both of these latter individuals had much more striking xanthomas than female homozygotes, and in the case of the homozygous brother of the proband, the hyperlipidemia was also more significant. The male homozygote had a VLDL cholesterol level that was at least 145 mg/dl higher than any female homozygote, suggesting that hormonal status may play a role in the expression of type III HLP associated with apoE deficiency.

Effective lipid-lowering therapy in homozygous apoE deficiency appears to be similar to that utilized for other forms of type III HPL (3). These patients' lipids were responsive to both dietary cholesterol and saturated fat restriction as well as clofibrate and nicotinic acid. Mean reductions in VLDL cholesterol of 53% of baseline values ($P < 0.01$, paired t test analysis), and increases in HDL cholesterol of 75% of baseline ($P < 0.01$) were achieved with administration of clofibrate at a dose of 1 g p.o. twice daily. Why such marked increases in HDL were achieved is not clear. However, the proband's plasma lipoproteins were not greatly reduced by estrogen treatment in contrast to reports in other type III HLP patients (38). More efficacy might have been achieved with a lower dose of estrogen. No evidence of any other systemic disease, neurologic deficit, or enhanced susceptibility to infection was noted in any homozygote, even though it has been suggested that apoE may play an important role in the physiology of nervous tissue and appears to be a major secretory protein of macrophages (39, 40).

While plasma lipid and lipoprotein cholesterol concentrations in 8 of 10 obligate heterozygous offspring of homozygotes were normal, VLDL cholesterol values were elevated in two offspring, and in the proband's mother and paternal uncle (possible heterozygote). Also an abnormal slowly migrating prebeta lipoprotein band was noted in two young normolipidemic obligate heterozygotes (offspring of proband) that were tested. A beta migrating VLDL band was only observed in homozygotes. These data indicate that heterozygotes may develop mild hyperlipidemia. The aging process and/or menopause may affect the expression of the hyperlipidemia in heterozygotes and homozygotes. By history the proband first developed xanthomas at age 50 yr.

Complete lipoprotein analysis indicates that homozygotes develop accumulations of all lipoprotein constituents within the VLDL and IDL density region (see Table II). These observations are consistent with data derived by analytical ultracentrifugation. Gradient gel electrophoresis and electron microscopy data on lipoproteins isolated from the proband indicate the presence of small, dense VLDL, and heterogeneous populations within IDL and LDL. In the LDL region there was a definite shift to larger less dense components than normal LDL (20). The HDL was normal in size and morphology (21).

Apolipoprotein quantitation indicated elevations of apolipoproteins A-I, A-II, and C-II in plasma, and a threefold increase in VLDL apoB in homozygotes as compared with normal. Only trace amounts of apoE were noted in the plasma of homozygotes by radioimmunoassay in our laboratory, while apoE was not detectable by electroimmunoassay in the laboratory of Dr. Alaupovic (Oklahoma Medical Research Foundation) or by radioimmunoassay in the laboratory of Dr. Conrad Blum (Columbia Presbyterian Hospital, New York). If apoE is present in homozygotes, its concentration is <0.5% of that observed in normals. Heterozygotes had mean plasma apoE concentrations that were ~50% of normal, indicating that this biochemical abnormality is expressed in a codominant fashion in these individuals. Why levels of apoA-I and apoA-II are somewhat increased in homozygotes is not clear. It should be noted that our control group for apolipoprotein analysis was Caucasian, while the study subjects were Black. Studies comparing Black and White subjects in terms of HDL cholesterol and apoA-I levels indicate that Blacks have slightly higher levels than Whites (32, 33). The increased HDL constituents observed therefore, may in part be due to racial differences. However, the levels of apoA-I and apoA-II in homozygotes were also higher than those reported for normal Black subjects (see Table III). ApoE deficiency therefore may be associated with a mild increase in HDL constituents. No other apolipoproteins were deficient in homozygotes since apoC-I, C-III, apoD, and apoF were all present in approximately normal amounts based on electroimmunoassay. In contrast to the linkage of apoA-I and apoC-III in the familial apoA-I and C-III deficiency states (17, 41–43), no other apolipoprotein deficiency appears to be linked to apoE deficiency. PAGE indicated the accumulation of apoB-48 and apoA-IV in VLDL, IDL, and LDL of homozygotes. These data suggest that chylomicron remnants accumulate in this condition.

Previous studies of apoE kinetics in normal subjects indicate synthesis rates of ~3–4 mg/kg per d, and plasma residence times of ~0.7 d (28). In addition, apoE2 is catabolized more slowly than apoE3 in both normal or type III HLP subjects (44). The data presented here indicate that apoE3 injected on the proband's lipoproteins was catabolized almost twice as rapidly as apoE3 injected on normal lipoproteins (see Table IV, Fig. 10). These data suggest that remnant lipoprotein particles containing apolipoproteins such as apoB-48 and apoA-IV, found in the proband's plasma but lacking in normal fasting plasma, may enhance the uptake of apoE. Alternatively, this difference may have been due to the different lipid content of the proband's VLDL. These data do indicate that the type of lipoprotein particle to which labeled apoE is bound can affect its clearance.

ApoE kinetics in the proband indicate markedly decreased production of apoE. It should be noted that the kinetics of normal apoE, and not the proband's apoE, were assessed in the proband. The kinetic data therefore are valid only if the proband synthesizes apoE that is structurally normal. Recent data examining apoE messenger (m)RNA levels in macrophages from homozygotes indicate only trace amounts of mRNA to be present as compared with normal macrophages, and an additional abnormal apoE mRNA band was also observed (45). The apoE gene was present in homozygotes, and no restriction fragment length polymorphism was noted (45, 46). Therefore, the defect in this genetic condition appears to be due to an inability to synthesize apoE and the precise defect at the gene level remains to be elucidated (45). The retarded catabolism of the apoE tracer in the apoE-deficient patient was probably due to lack of a sufficient number of apoE molecules per triglyceride-rich particle to result in a normal catabolic rate, since apoE was present in only tracer amounts.

The results of apolipoprotein B kinetic studies indicate that unlike apoB-100, very little apoB-48 radioactivity (~1%) is transferred from VLDL to LDL consistent with previous reports in rats and man (27, 47, 48). It has been reported that apoB-48 fractional catabolism is slower than that of apoB-100 in severely

hypertriglyceridemic subjects similar to our finding in the proband (49). Our data also indicate that apoB-48 is catabolized at a significantly faster rate than apoB-100 in normal subjects consistent with the previous data (47–50). ApoE deficiency results in a much more striking impairment of VLDL apoB-48 catabolism than VLDL apoB-100 catabolism, similar to observations in other hypertriglyceridemic subjects (50). The calculated VLDL apoB-100 synthesis rate for the proband was normal. It is possible that such differences could partially be explained by differences in the composition and density of apoB-48 and apoB-100 containing lipoproteins. This would not explain, however, the marked differences between kinetic parameters for apoB-48 and apoB-100 observed in the proband versus the normal subject, since an identical tracer was injected. In addition, LDL apoB-100 catabolism in the proband appears to be similar to normal. Gel electrophoresis data demonstrate the accumulation of apoB-48 and apoA-IV in the VLDL, IDL, and LDL of homozygotes. These data suggest that apoE is essential for the normal receptor-mediated catabolism of triglyceride-rich lipoprotein remnants (50, 51). Moreover, apoB-48 and/or apoA-IV may also play a role in the uptake of triglyceride-rich lipoproteins, since apoE was catabolized at a significantly greater fractional catabolic rate on lipoproteins isolated from the proband than on normal lipoproteins when studied in normal subjects.

The combined data are consistent with the concepts that familial apoE deficiency is a rare autosomal recessive condition associated with type III hyperlipoproteinemia and markedly decreased apoE production, and that apoE is essential for the normal catabolism of triglyceride-rich lipoprotein remnants.

Acknowledgments

We wish to thank Leslie L. Jenkins for technical assistance; Dr. Nicholas P. Papadapoulos, Clinical Chemistry, National Institutes of Health for performing the agarose lipoprotein electrophoresis; Dr. Conrad Blum, Department of Medicine, Columbia Presbyterian Hospital, New York, NY, for performing the apoE immunoassay; and Dr. Petar Alaupovic, Oklahoma Medical Research Foundation, Oklahoma City, OK, for performing immunoassays. We wish to thank Dr. Jimmy Light of the Renal Transplant Service, Walter Reed Army Medical Center, Washington, DC, and Dr. Hugh Willis and Dr. William Lorimer of Chatham, VA for allowing us to study their patients.

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