Upregulated Synthesis of Both Apolipoprotein A-I and Apolipoprotein B in Familial Hyperalphalipoproteinemia and Hyperbetalipoproteinemia

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A family was identified with vertical transmission through three generations with simultaneous increases of apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), low-density lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol, which we have designated familial hyperalphalipoproteinemia and hyperbetalipoproteinemia (HA/HBL). Affected patients develop xanthomas and coronary artery disease (CAD). HA/HBL apoA-I and LDL-apoB were isolated and characterized. The in vivo kinetics of radiolabeled apoA-I and LDL-apoB were evaluated in two HA/HBL probands and three controls. Structural and metabolic characterization showed normal apoA-I and LDL-apoB. The kinetics of metabolism of HA/HBL apoA-I in the HA/HBL subjects showed that elevated apoA-I levels were solely due to an increased synthesis rate (15.2 to 17.6 mg/kg/d *v* 11.1 to 11.4 mg/kg/d) with a normal apoA-I residence time in plasma (4.2 to 5.4 days *v* 5.1 to 5.3 days). The elevation of LDL-apoB levels resulted from both an increased synthetic rate (16.6 to 22.9 mg/kg/d *v* 12.3 to 13.8 mg/kg/d) and a prolonged residence time (3.3 to 3.8 days *v* 1.4 to 1.9 days). In addition, we evaluated another HA/HBL proband of an unrelated family with HA/HBL to confirm the kinetic data. LDL-receptor binding studies of HA/HBL fibroblasts showed normal binding, uptake, and degradation of LDL isolated from a normolipemic control. The serum concentration of the cholesterol ester transfer protein (CETP) was normal in the studied probands. An apoB 3500 and apoB 3531 mutant, respectively, was ruled out by polymerase chain reaction (PCR). In conclusion, the site of the molecular defect in HA/HBL subjects may be involved in the coordinate regulation of metabolism for both LDL and HDL.

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SEVERAL RISK FACTORS that cause premature coronary artery disease (CAD) have been identified.[1-5] Increased levels of low-density lipoprotein (LDL)-cholesterol (hyperbetalipoproteinemia) represent a potent risk factor for atherogenesis.[6] An example for familial aggregation of elevated LDL-cholesterol is familial hypercholesterolemia, which is due to a deficiency of the LDL receptor.[7,8] The genetic trait is autosomal dominant. The incidence for the heterozygous state is approximately 1:500, and for the homozygous state, 1:1,000,000. The clinical phenotype of heterozygous patients is variable. However, untreated patients usually have first signs of atherosclerotic lesions between the age of 35 and 45 years. In contrast, homozygous patients have onset of atherosclerosis as early as in childhood. Untreated patients commonly experience myocardial infarction before 20 years of age. Another cause for inherited severe hypercholesterolemia is a defective binding of the LDL particle to the LDL receptor, which results from a guanine to adenine transition in codon 3500 of the apolipoprotein B (apoB) gene. This mutation leads to an arginine to glutamine exchange in the putative receptor binding region of apoB-100.[9] Recently, an additional mutation was characterized, the $Arg_{3531} \rightarrow Cys$ mutation due to a C → T transition at nucleotide 10800, which also causes familial ligand-defective apoB-100.[10]

In contrast to hyperbetalipoproteinemia, increased levels of serum high-density lipoprotein (HDL)-cholesterol (hyperalphalipoproteinemia) are inversely correlated with CAD.[11-15] The HDL particle plays an important role in the transport of cholesterol from the peripheral cells to the liver, the so-called reverse cholesterol transport.[16] There are reports of familial aggregation of hyperalphalipoproteinemia with late onset of atherosclerosis and longevity.[17-22] However, there are also patients with hyperalphalipoproteinemia who present no delayed onset of CAD and no increased life expectancy.[23]

It is unusual to have the simultaneous expression of elevated serum concentrations of LDL-cholesterol and HDL-cholesterol (hyperalphalipoproteinemia and hyperbetalipoproteinemia) in the same individual. We have identified a family with familial hyperalphalipoproteinemia and hyperbetalipoproteinemia (HA/HBL), which is transmitted vertically through three generations (Fig 1). The index patient in this family presented xanthomas and premature CAD.

Assuming that the primary defect corresponds to the metabolism of the apolipoproteins, we investigated the in vivo metabolism of apoA-I and LDL-apoB in three probands with hyperalphalipoproteinemia and hyperbetalipoproteinemia to determine the metabolic etiology of HA/HBL.

SUBJECTS AND METHODS

Study Subjects

The first identified person of this family (Fig 1) was a 65-year-old woman (HA/HBL 1) with a history of angina, cutaneous and tendinous xanthomas, and arcus lipoides corneae starting at age 45. No other atherogenic risk factors have been identified. She underwent triple aortocoronary bypass at the age of 57, because of severe CAD as documented angiographically (multiple stenosis > 75%). Her 46-year-old daughter (HA/HBL 2) has tendon xanthomas, arcus lipoides corneae, and sonographically documented plaques of the aorta. Her 20-year-old grandson (HA/HBL 3) is clinically free of symptoms. HA/HBL 3 did not participate in the metabolic study.

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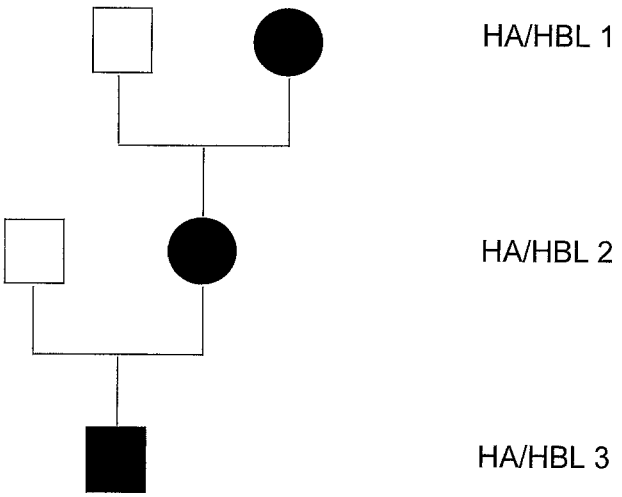

Fig 1. Pedigree of the HA/HBL family. The index patient was a 65-year-old woman (HA/HBL 1); her 46-year-old daughter (HA/HBL 2) and her 20-year-old grandson (HA/HBL 3) were also affected. HA/HBL subjects are indicated by solid symbols.

Metabolic Study

Five subjects, three normal volunteers and two patients with familial HA/HBL (HA/HBL 1 + 2), participated in a metabolic study. Subsequently, another proband with HA/HBL (HA/HBL 4) of an unrelated family with familial HA/HBL and two additional normal volunteers were studied. They were hospitalized for the study in the Clinical Center of the National Institutes of Health. None of the subjects had hepatic, hematologic, or renal abnormalities. None had a severe disease. The subjects were not on any medication that could interfere with lipid metabolism. All subjects gave informed consent. The study protocol was approved by the Human Use Research Committee of the National Heart, Lung, and Blood Institute.

Isolation of ApoA-I and LDL

After ultracentrifugation, isolated HDL (*d* 1.063 to 1.21 g/mL) was dialyzed and delipidated with chloroform/methanol 2:1 (vol/vol).[24] ApoA-I was separated from the other apolipoproteins by heparin affinity and Sephacryl S-200 gel permeation chromatography (Pharmacia, Uppsala, Sweden).[25] Purified apoA-I was a discrete electrophoretic band on sodium dodecyl sulfate (SDS; 15% acrylamide) and urea (pH 8.4) polyacrylamide gel electrophoresis (PAGE), using a modification of the method of Weber and Osborn.[26,27] LDL was isolated between *d* 1.019 and *d* 1.063 g/mL, purified by recentrifugation for 22 hours at *d* 1.070, and dialyzed and concentrated against 50 mmol/L sodium phosphate 100 mmol/L saline (pH 7.4).

Iodination of ApoA-I and LDL

The prepared samples were sterilized by filtration through a 0.22-μm filter and then radioiodinated by a modification of the iodine monochloride method.[28,29] A 100-μg quantity of lyophilized apoA-I was redissolved in 50 μL buffer of 6 mol/L guanidine-HCl (pH 8.5). One milliliter of the prepared LDL solution was added to a 1 mL solution of 1 mol/L glycine (pH 10). Then, 5 mCi of ^{125}I and ^{131}I, respectively, was added to each solution, followed by the slow addition of iodine monochloride. The quantity of ICl added was calculated to yield 1 mol of iodine monochloride. The efficiencies of the iodinations were 20% to 40% for apoA-I and 7% to 40% for LDL. Radiolabeled apoA-I was incubated with plasma from a normal volunteer for 30 minutes at 37°C, and the density of the plasma adjusted to 1.21 g/mL with solid potassium bromide (KBr) and centrifuged at 59,000 rpm. The *d* 1.21 g/mL supernatant was isolated by tube slicing. Each sample was dialyzed against 50 mmol/L sodium phosphate/100 mmol/L saline (pH 7.4), sterilized by filtration through a 0.22-μm filter and tested for pyrogens before injection into the study subjects. Between 96.1% and 99.6% of the total radioactive iodine was bound to protein after precipitation with trichloric acid. The distribution of radioiodine between the proteins of injected apoA-I and LDL was determined by preparative PAGE using 15% acrylamide gels. A total of 94.8% and 94.9%, respectively, of the total radioactivity was running with apoA-I and 78.6 to 83.3% of the total radioactivity was running with apoB.

Study Protocol

Study subjects were placed on an isoweight diet. Caloric intake was 42% carbohydrates, 42% fat, 16% protein, 200 mg of cholesterol per 1,000 kcal, and a polyunsaturated/saturated fat ratio of 1:3, leading to minimal variation in plasma cholesterol, triglyceride, and apolipoprotein concentrations. Two days before injection, subjects were started on potassium iodide (1,200 mg/d). Subjects were injected intravenously with up to 25 μCi of ^{131}I and 15 μCi of ^{125}I. Blood samples were obtained at 10 minutes, 1, 3, 6, 12, 24, and 36 hours, and on days 2, 3, 5, 7, 9, 11, and 14; samples were collected into tubes containing EDTA at a final concentration of 0.01%, stored at 4°C, and centrifuged (2,000 rpm, 30 minutes) at 4°C. Aprotinin and Na-azide were added to each plasma sample at a final concentration of 0.05% and 200 Kallikrein inhibitor units/mL. The plasma lipoproteins were isolated by ultracentrifugation; the radioactivities in plasma and the lipoproteins subfractions were quantitated in a Packard auto gamma counter (Packard Instrument, Meriden, CT) and their apoA-I and apoB concentrations determined.

Analytical Methods

Plasma cholesterol and triglycerides were measured on an enzymic analyzer (Gilford System 3500; Gilford Instruments, Oberlin, OH). HDL-cholesterol was determined in plasma after dextran sulfate precipitation.[30] The remaining lipid and lipoprotein analyses were performed using the methods of the Lipid Research Clinics.[31] ApoA-I and apoB concentrations were determined by radial immunodiffusion.[32,33] Measurement of the cholesterol ester transfer protein (CETP) mass was performed using a radioimmunoassay.[34]

The residence time (1/fractional catabolic rate) was calculated from the area under the multiexponential plasma decay curve by a multiexponential computer curve-fitting technique, using the SAAM Manual.[35] The production rate was determined by dividing the pool size by the residence time. The pool size equals the apolipoprotein concentration multiplied by the plasma volume per kilogram body weight. The plasma volume is determined by dividing the total quantity injected by the radioactivity per unit volume determined in the sample obtained 10 minutes after injection.

DNA Preparation

Heparinized blood was lysed with 0.32 mol/L sucrose/10 mmol/L $MgCl_2$/1% Triton X-100 at 4°C. The nuclei were collected by centrifugation at 1,000 × *g* for 10 minutes. The nuclear pellet was suspended in 0.075 mol/L NaCl/0.024 mol/L EDTA (pH 8.0). Proteinase K at 2 mg/mL and 5% SDS were added. The mixture was incubated for 12 hours at 37°C. The DNA was extracted by using Tris-HCl saturated phenol and chloroform/isoamyl alcohol (24:1, vol/vol). The DNA was precipitated using 3 mol/L sodium acetate and 100% ethanol.

ApoB Gene Analysis

Presence for the $arginine_{3500} \rightarrow$ glutamine and $arginine_{3531} \rightarrow$ cysteine mutations were investigated by polymerase chain reaction

Table 1. Lipoprotein Characteristics of Studied Subjects

Subject	Age (yr)/Sex	Weight (kg)	Lipoprotein Characteristic (mg/dL)					
			TC	TG	LDL-C	HDL-C	apoA-I	apoB
HA/HBL 1	63/F	67.2	407	136	237	111	235	192
HA/HBL 2	42/F	68.9	411	244	304	65	160	222
HA/HBL 3	19/M	68.2	336	192	211	89	230	186
HA/HBL 4	44/F	60.8	396	97	275	71	217	218
Normal 1	20/M	76.0	144	65	96	39	101	95
Normal 2	22/M	73.2	147	93	78	45	115	98
Normal 3	26/F	67.7	227	104	155	50	146	103
Normal 4	22/F	60.4	150	134	76	58	127	82
Normal 5	20/F	71.1	203	183	111	72	145	100
Range	20-40/M, F		185 ± 39	114 ± 56	118 ± 36	50 ± 14	117 ± 17	85 ± 19
Mean ± SD	40-60/M, F		209 ± 39	118 ± 52	136 ± 32	47 ± 18	120 ± 24	94 ± 26

Abbreviations: TC, total cholesterol; TG, triglycerides; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; F, female; M, male.

(PCR), followed by digestion with the restriction enzymes *Msp*I and *Nsi*I, respectively.[36,37]

LDL-Receptor Binding

LDL-receptor analysis was performed by a modification of the method of Goldstein and Brown.[38,39] Skin fibroblasts were incubated with ^{125}I-labeled LDL in 24-well plates (10,000 cells/well). Lipoprotein-deficient serum was obtained by recalcified human plasma from normal volunteers.

RESULTS

The clinical characteristics and lipid and lipoprotein values for HA/HBL 1 to 4 and the normal control subjects are summarized in Table 1. The values for total cholesterol, triglycerides, LDL- and HDL-cholesterol, apoA-I, and apoB were calculated as mean values during the metabolic study, with the exception of HA/HBL 3. This patient did not participate in the kinetic study. The total cholesterol level was elevated for all four HA/HBL individuals when compared with an age- and sex-matched control group. In all four patients, both LDL- and HDL-cholesterol were elevated. In addition, there was an elevation of apoA-I and apoB levels compared with a matched control group. The increased triglycerides in HA/HBL 2 were probably inherited from her 66-year-old father, who had type IV hyperlipidemia (serum total cholesterol, 241 mg/dL; triglycerides, 344 mg/dL; LDL-cholesterol, 159 mg/dL; very–low-density lipoprotein [VLDL]-cholesterol, 45 mg/dL; HDL-cholesterol, 37 mg/dL; apoA-I, 114 mg/dL; apoB, 134 mg/dL). The 48-year-old father of HA/HBL 3 had a normal lipoprotein profile (serum total cholesterol, 198 mg/dL; triglycerides, 129 mg/dL; LDL-cholesterol, 123 mg/dL; VLDL-cholesterol, 22 mg/dL; HDL-cholesterol, 43 mg/dL; apoA-I, 112 mg/dL; apoB, 97 mg/dL). The relative composition of the lipoprotein subfractions in HA/HBL 1 to 4 showed normal values for the percentage of cholesterol esters (Table 2). The serum concentrations of CETP were within the normal range (1.8 to 2.9 μg/mL for HA/HBL 1 to 4). Twenty normolipemic adults served as a control for the CETP serum concentration (range, 1.7 to 2.7 μg/mL). All studied HA/HBL patients responded to treatment with lovastatin (Table 3). While the LDL-cholesterol level was remarkably reduced in HA/HBL 1, 2, and 4, there was no significant increase or decrease of HDL-cholesterol in HA/HBL 1 to 4.

We have investigated the in vivo metabolism of apoA-I and LDL-apoB in HA/HBL 1 and 2 to determine the metabolic etiology of familial HA/HBL. To determine whether the LDL isolated from a HA/HBL individual is metabolized normally, both normal and HA/HBL LDL were injected into a normal subject (Fig 2). As demonstrated here, HA/HBL LDL is catabolized at the same rate as the normal LDL, demonstrating that these LDL particles are metabolically normal. To compare the metabolism of LDL in a HA/HBL patient with that of a normal subject, autologous LDL was injected in both, and its catabolism studied. Figure 3 illustrates the metabolism of autologous LDL in normal and HA/HBL subjects. The HA/HBL patient has a decreased fractional catabolic rate for LDL compared with the normal subject.

LDL-apoB kinetic parameters are summarized in Table 4. The apoB plasma concentration was markedly elevated in the HA/HBL patients. The normal range for apoB in our laboratory

Table 2. Lipoprotein Composition Analysis of the Studied Patients

Variable	HA/HBL 1	HA/HBL 2	HA/HBL 3	HA/HBL 4	Controls* (90th percentile)
TC (mg/dL)	464	483	308	328	142-219
TG (mg/dL)	151	300	228	62	60-165
VLDL-C (mg/dL)	22	65	35	2	5-20
CE (%)	48	48	40	68	40-65
TG (mg/dL)	106	259	192	11	29-100
IDL-C (mg/dL)	6	16	7	4	2-16
CE (%)	66	70	76	60	46-75
TG (mg/dL)	6	4	2	7	6-27
LDL-C (mg/dL)	311	338	178	224	61-146
CE (%)	69	70	69	70	60-76
TG (mg/dL)	27	23	22	28	15-38
HDL_2-C (mg/dL)	81	29	42	59	13-30
CE (%)	70	71	70	72	60-82
TG (mg/dL)	5	5	4	8	3-19
HDL_3-C (mg/dL)	41	33	43	37	20-36
CE (%)	78	79	78	80	74-88
TG (mg/dL)	4	6	6	5	5-22
VHDL-C (mg/dL)	3	2	3	2	2-4
CE (%)	63	63	47	59	34-64
TG (mg/dL)	3	3	2	3	2-15

Abbreviations: very–high-density lipoprotein-cholesterol; CE, cholesterol esters.

*120 healthy control subjects.

Table 3. Drug Response in the Studied Patients

Subject	Lovastatin (mg/d) 0	20	40
HA/HBL 1			
TC (mg/dL)	464	329	304
LDL-C (mg/dL)	317	183	151
HDL-C (mg/dL)	125	128	135
HA/HBL 2			
TC (mg/dL)	483	331	286
LDL-C (mg/dL)	355	214	175
HDL-C (mg/dL)	64	65	79
HA/HBL 3			
TC (mg/dL)	283	298	264
LDL-C (mg/dL)	170	175	148
HDL-C (mg/dL)	80	104	87
HA/HBL 4			
TC (mg/dL)	339	257	261
LDL-C (mg/dL)	240	155	175
HDL-C (mg/dL)	97	102	81

is 85 ± 19 mg/dL. The residence times for apoB catabolism were prolonged 1.5-fold to twofold in the study subjects and the production rates for apoB were also markedly elevated. Therefore, elevated apoB plasma concentrations in these HA/HBL probands are due to both increased production rate and prolonged residence time.

To investigate whether the apoA-I isolated from a HA/HBL individual is metabolized normally, both normal and HA/HBL apoA-I were injected into a normal subject (Fig 4). The plasma apoA-I decay curves are superimposable, demonstrating a normal catabolic rate of the HA/HBL apoA-I. We have obtained identical results in another normal subject studied. In addition, we have performed two-dimensional gel electrophoresis and amino acid analysis on the apoA-I from the HA/HBL individuals. This apoA-I, when compared with normal apoA-I, had a normal isoelectric point, normal relative distribution of apoA-I isoforms, and normal amino acid composition (Table 5). Therefore, in addition to being metabolically normal, the

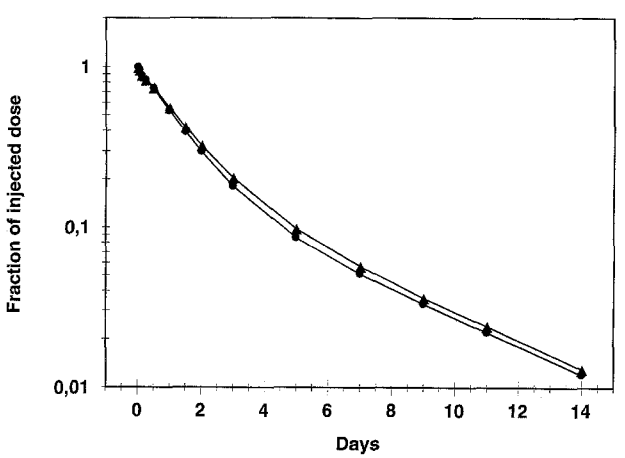

Fig 2. Metabolism of normal and HA/HBL LDL in a normal subject. The ordinate is the fraction of injected dose on a log scale. The abscissa is the time in days on a linear scale. Plasma decay curves as fraction of the injected dose after simultaneous injection of ^{131}I-labeled LDL from a normal subject (●) and ^{125}I-labeled LDL from HA/HBL 1 (▲) into a normal control subject.

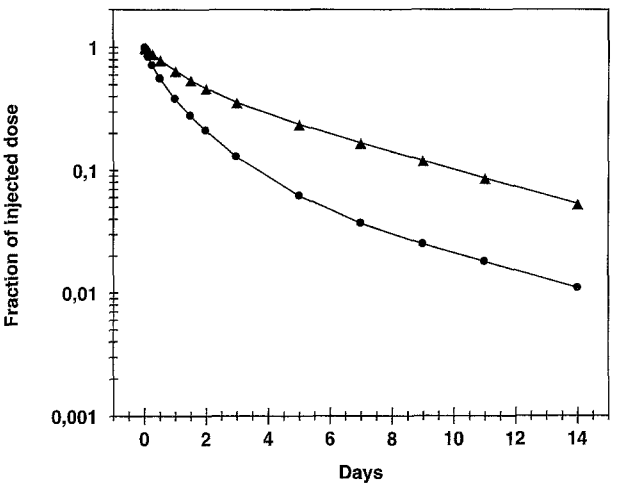

Fig 3. Metabolism of autologous LDL in normal and HA/HBL subjects. The ordinate is the fraction of injected dose on a log scale. The abscissa is the time in days on a linear scale. Comparison of the plasma decay curves as fraction of the injected dose after injection of autologous HA/HBL ^{125}I-labeled LDL into HA/HBL 1 (▲) and normal ^{131}I-labeled LDL into a normal subject (●).

apoA-I from the individuals with HA/HBL is structurally normal.

To investigate how a patient with HA/HBL metabolizes apoA-I, the autologous apoA-I was injected into a normal subject and a HA/HBL subject (Fig 5). Again, we see two superimposable decay curves, demonstrating a normal catabolic rate of apoA-I in the study subject. Again, similar results were obtained in the other HA/HBL subjects. In Table 6, the kinetic parameters of apoA-I metabolism are summarized. As mentioned earlier, the HA/HBL subjects have increased apoA-I levels. The normal control has a high normal apoA-I level of 146 mg/dL, while both of the HA/HBL patients have frankly elevated apoA-I levels. The apoA-I residence times and the fractional catabolic rate of the study subjects and controls were not significantly different, while the production rate of apoA-I in the HA/HBL subject was increased by greater than 50% compared with the normal control. In addition to the control subject studied here, the kinetics of apoA-I metabolism were studied in 15 other normal subjects. We found that apoA-I residence times vary between 4 and 6 days, with production rates ranging from 9 to 11 mg/kg/d. Therefore, the elevated apoA-I level in the HA/HBL subjects is due to an increased rate of synthesis.

To rule out a defect in the LDL receptor, we investigated LDL

Table 4. Kinetic Parameters of LDL-apoB Metabolism

Subject	Plasma apoB (mg/dL)	RT (d)	FCR (1/d)	PR (mg/kg/d)
HA/HBL 1	192	3.6	0.28	16.6
HA/HBL 2	222	3.3	0.30	20.2
HA/HBL 4	218	3.8	0.26	22.9
Normal 1	95	1.9	0.53	12.3
Normal 2	98	1.8	0.55	13.6
Normal 4	82	1.4	0.71	13.8

Abbreviations: RT, residence time; FCR, fractional catabolic rate; PR, production rate.

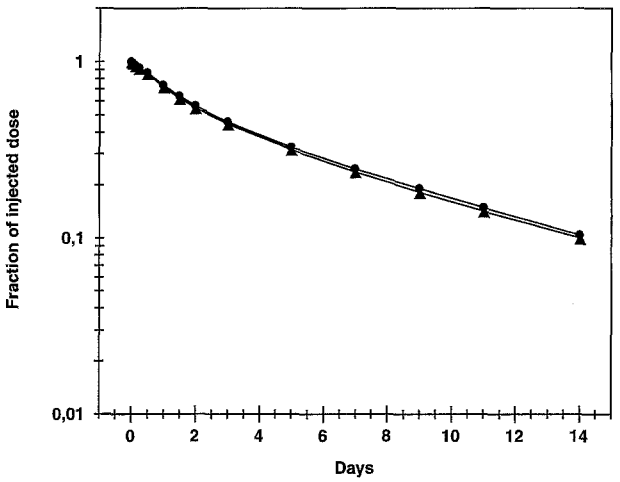

Fig 4. Metabolism of normal and HA/HBL apoA-I in a normal subject. The ordinate is the fraction of injected dose on a log scale. The abscissa is the time in days on a linear scale. Plasma decay curves as fraction of the injected dose after simultaneous injection of ^{125}I-labeled apoA-I from a normal control (●) and ^{131}I-labeled apoA-I from HA/HBL 1 (▲) into a normal subject.

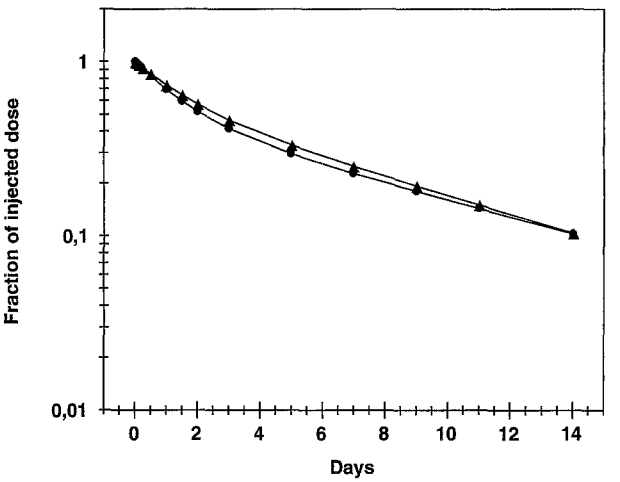

Fig 5. Metabolism of autologous apoA-I in normal and HA/HBL subjects. The ordinate is the fraction of injected dose on a log scale. The abscissa is the time in days on a linear scale. Comparison of the plasma decay curves as fraction of the injected dose after injection of autologous HA/HBL ^{131}I-labeled apoA-I into HA/HBL 1 (▲) and normal ^{125}I-labeled apoA-I into a normal subject (●).

binding on HA/HBL fibroblasts, which showed completely normal binding, uptake, and degradation of normal LDL by fibroblasts isolated from HA/HBL 1 to 3 (Fig 6; degradation data not shown). Therefore, we have no evidence for a delayed catabolism of LDL due to a LDL-receptor deficiency. In addition, we excluded both an apoB 3500 defect and an apoB 3531 defect by PCR for all four studied HA/HBL probands.

DISCUSSION

Hyperlipoproteinemia is frequently associated with premature atherosclerotic cardiovascular disease. There are many genetic diseases that result in hyperlipoproteinemia. Hyperalphalipoproteinemia correlates with a decreased risk for CAD, while hyperbetalipoproteinemia is associated with an increased risk. It is unusual to have the simultaneous expression of hyperalphalipoproteinemia and hyperbetalipoproteinemia in the same individual. We have identified a family with familial hyperalphalipoproteinemia and hyperbetalipoproteinemia, which was transmitted vertically through three generations. The index patient of this family has xanthomas and premature CAD. The primary defect of this lipid disorder is unknown. Exogenous causes such as sex and age dependency can be excluded. The family members live in different areas and have different dietary habits. To explain the vertical transmission in this family with HA/HBL, a monogenic autosomal-dominant inheritance is possible, as it is assumed for familial hyperalphalipoproteinemia.[17-20,40,41] However, two or more defects may also result in this phenotypic expression, especially if they are closely linked to each other. However, close linkage of one locus for hyperalphalipoproteinemia and a second locus for hyperbetalipoproteinemia is not likely, because of the rare existence of both forms of hyperlipidemia within the same patient.

Decreased activity of CETP, increased activity of lecithin: cholesterol acyltransferase, and lipase deficiency have been reported to result in hyperalphalipoproteinemia.[42-47] The normal lipid and apolipoprotein composition of HDL and LDL in our HA/HBL probands is compatible with a normal CETP and lecithin:cholesterol acyltransferase activity. The postheparin lipolysis activity and the serum concentration of CETP were within the normal range in our probands. The relative amino

Table 5. Amino Acid Composition of Normal and HA/HBL apoA-I

Amino Acid	Predicted Value	Normal apoA-I	HA/HBL 1 apoA-I	HA/HBL 2 apoA-I	HA/HBL 4 apoA-I
Asp	21	21.2	21.5	21.3	21.4
Thr	10	10.0	9.8	9.8	9.8
Ser	15	14.9	14.7	14.7	14.7
Glu	46	46.8	47.0	46.7	46.9
Pro	10	10.6	10.4	10.4	10.4
Gly	10	10.8	10.6	10.4	10.5
Ala	19	19.6	19.8	19.7	19.8
Val	13	11.8	11.8	11.7	11.7
Met	3	2.9	3.0	2.9	2.9
Ile	0	0.3	0.1	0.0	0.1
Leu	37	36.9	37.7	37.6	37.6
Thy	7	7.0	7.0	7.0	7.0
Phe	6	6.2	6.2	6.2	6.2
His	5	4.7	4.8	4.8	4.8
Lys	21	20.2	20.6	20.4	20.6
Arg	16	16.7	17.0	16.9	16.9

Table 6. Kinetic Parameters of apoA-I Metabolism

Subject	Plasma apoA-I (mg/dL)	RT (d)	FCR (1/d)	PR (mg/kg/d)
HA/HBL 1	235	5.4	0.19	17.6
HA/HBL 2	160	4.2	0.24	15.2
HA/HBL 4	217	5.4	0.19	16.1
Normal 3	146	5.3	0.19	11.1
Normal 5	145	5.1	0.20	11.4

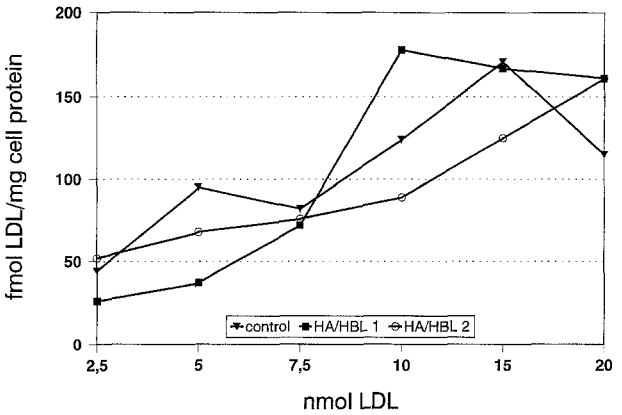

Fig 6. LDL-receptor binding analysis. Fibroblasts were obtained by in vitro culture of a skin biopsy from HA/HBL 1 and HA/HBL 2 and a normal control subject. Binding and uptake of ^{125}I-labeled normal LDL of the skin fibroblasts from both studied subjects are illustrated. Data represent the mean of quadruplicate assays. In addition, we observed normal degradation (data not shown).

acid composition of apoA-I was shown to be normal in HA/HBL. Kinetic data showed a metabolically normal HA/HBL apoA-I. The elevated apoA-I serum concentration in HA/HBL subjects is solely due to an increased synthetic rate of apoA-I. This phenomenon has been reported previously in a patient with familial hyperalphalipoproteinemia.[48] Therefore, this is the second report to confirm an upregulation of apoA-I synthesis in patients with hyperalphalipoproteinemia. The LDL isolated from these subjects had a normal composition and was metabolically normal. The elevated LDL levels are due both to an increased synthetic rate and to a decreased fractional catabolic rate. Subsequently, a defect in the LDL receptor was ruled out on the basis of the fibroblast LDL binding, and a defect of the apoB 3500 gene was excluded by PCR.

The chromosomal localization of this lipoprotein disorder remains to be elucidated. Monge et al. did show that in HepG 2 cells, there is a coordinate regulation of apoA-I and apoB-100 synthesis in vitro.[49,50] Cells were incubated with human LDL. The higher the LDL concentration, the higher the apoA-I mRNA expression and the lower the apoB mRNA expression. Therefore, we propose that the site of the molecular defect in familial HA/HBL may be involved in the coordinate regulation of both classes of lipoproteins.

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