Apolipoprotein A-I metabolism in subjects with a PstI restriction fragment length polymorphism of the apoA-I gene and familial hypoalphalipoproteinemia[1]

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Abstract Familial hypoalphalipoproteinemia (hypoalpha), characterized by a decreased high density lipoprotein level, is associated with an increased incidence of premature cardiovascular disease. Restriction fragment length polymorphism analysis of genomic DNA has detected a polymorphism for the PstI restriction endonuclease near the apoA-I gene, with either a 2.2 or a 3.3 kb fragment. The latter has been previously found to occur with significantly higher frequency in probands of families with familial hypoalpha. ApoA-I was isolated from three unrelated subjects with familial hypoalpha and the 3.3 kb PstI polymorphism of the apoA-I gene, and from normal control subjects. The apoA-I from the hypoalpha subjects was structurally normal as determined by amino acid analysis and by two-dimensional gel electrophoresis. When normal apoA-I and hypoalpha apoA-I were simultaneously injected into either normal controls or hypoalpha subjects, both forms of apoA-I were catabolized at the same rate in the same subject, indicating that the hypoalpha apoA-I is also metabolically normal. Analysis of the kinetics of metabolism of apoA-I in the hypoalpha subjects, compared to the normal controls, revealed that the reduced plasma levels of apoA-I were due to an increased apoA-I fractional catabolic rate, and that the synthetic rate was normal. Based on these results, we conclude that the apoA-I gene in these hypoalpha subjects is normal, and the PstI polymorphism near the apoA-I gene, which is associated with familial hypoalpha, is likely to be a marker for a mutant gene closely linked to, but not in, the apoA-I gene. —**Roma, P., R. E. Gregg, C. Bishop, R. Ronan, L. A. Zech, M. V. Meng, C. Glueck, C. Vergani, G. Giudici, and H. B. Brewer, Jr.** Apolipoprotein A-I metabolism in subjects with a PstI restriction fragment length polymorphism of the apoA-I gene and familial hypoalphalipoproteinemia. *J. Lipid Res.* 1990. **31:** 1753–1760.

Supplementary key words high density lipoproteins • residence time

High density lipoproteins are a heterogeneous class of lipoproteins with alpha electrophoretic mobility and a density range of 1.063–1.210 g/ml (2). HDL have been intensively investigated in order to better understand their metabolic function and to determine the mechanism by which they modulate cholesterol metabolism. In vitro studies have demonstrated that intracellular accumulation of cholesterol up-regulates the binding of HDL to specific sites on the plasma membrane of fibroblasts (3) and macrophages (4), and that HDL have the ability to facilitate the egress of cholesterol from cultured cells (5). Epidemiologic studies have demonstrated that HDL cholesterol is inversely correlated with the incidence of cardiovascular disease (6–8). In addition, the plasma concentration of apoA-I, the major HDL apolipoprotein, is a negative risk factor for the development of coronary artery disease (9–11).

Families have been described in which hypoalphalipoproteinemia (hypoalpha) is inherited in a dominant fashion (12, 13). In these reports, familial hypoalphalipoproteinemia is characterized by normal plasma levels of triglycerides, cholesterol, VLDL, and LDL, with an HDL cholesterol below the tenth percentile, and affected individuals have an increased incidence of premature cardiovascular disease. It has been speculated that abnormalities in the apoA-I gene may be causally related to familial hypoalpha.

The mature form of apoA-I is a 243 amino acid long polypeptide that is synthesized as a preproapolipoprotein (14, 15). The apoA-I gene is composed of four exons and three introns (16–18) and is part of a multigene complex

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoprotein; hypoalpha, hypoalphalipoproteinemia; RFLP, restriction fragment length polymorphism; PBS, phosphate-buffered saline; RT, residence time.

[1]Preliminary results of these studies have been presented in abstract form at the AAP/ASCI/AFCP National Meeting, 1–4 May 1987, San Diego, CA (1).

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with the genes for apoC-III and apoA-IV on the long arm of chromosome 11 (18). Restriction endonucleases have been used to detect polymorphic sites in the A-I, C-III, A-IV apolipoprotein gene cluster, and detailed restriction endonuclease fragment maps of the apoA-I gene have been constructed (17–19). A PstI restriction endonuclease site 3' to the polyadenylation signal of the human apoA-I gene is polymorphic, giving rise to a 2.2 kb and a less frequent 3.3 kb fragment. Sidoli et al. (20) performed restriction fragment length polymorphism analysis in twelve members of the hypoalpha family described by Vergani and Bettale (12) and found a linkage of the apoA-I gene PstI+/SacI− haplotype with the phenotype of hypoalphalipoproteinemia. Furthermore, the frequency of the 3.3 kb PstI fragment has been reported to be significantly increased in the probands of families with familial hypoalpha and premature cardiovascular disease (21), 2% in the control population versus 42% in the familial hypoalpha subjects. Therefore, the PstI 3.3 kb long fragment has been proposed to be a genetic marker for an as yet unidentified mutant gene that results in hypoalphalipoproteinemia, with apoA-I gene being the most likely candidate. We have studied the metabolism of apoA-I in three unrelated subjects who had familial hypoalpha and the 3.3 kb PstI polymorphism in order to understand the genetic basis of their decreased apoA-I levels, and to establish whether any abnormality of the apoA-I gene is responsible for their dyslipoproteinemia.

MATERIALS AND METHODS

Patients and controls

ApoA-I kinetic studies were performed in three patients with familial hypoalpha with the 3.3 kb PstI RFLP and in seven control subjects. The control subjects were normolipidemic volunteers without evidence of coronary artery disease, or acute or chronic illnesses. Patient number 1 has not been previously described. He is asymptomatic, and his low HDL cholesterol was first noted on routine screening. The proband has a positive family history for premature coronary artery disease and sudden death. His father died at the age of 31 of a myocardial infarction; however, there is no available information on his plasma lipids or lipoproteins. The subject's paternal uncle has hypoalpha and had coronary artery bypass grafting at the age of 45. On the maternal side of the family, his great grandfather and great uncle died at the ages of 27 and 51, respectively, of myocardial infarctions. Patient number 2, whose father died of a myocardial infarction at the age of 35, has diffuse coronary artery disease. He is subject III-6 in the family described by Vergani et al. (12). Patient number 3 is the asymptomatic proband (subject II-2) of family 14 in the study of Third et al. (13).

The study protocol was approved by the Institutional Review Board, National Heart, Lung, and Blood Institute, and written informed consent was obtained from each subject. Patients and control subjects were within 10% of ideal body weight and were on an ad libitum diet before admission to the metabolic ward of the Clinical Center at the National Institutes of Health. None of the study subjects were on any medications, abused alcohol or other drugs, and only patient number 2 smoked, and he smoked two packs of cigarettes per day. Three days prior to the start of the experiment the subjects were placed on an isocaloric diet that consisted of 16% protein, 42% carbohydrate, 42% fat, polyunsaturated to saturated fat ratio of 0.2, and 200 mg cholesterol/1000 kcal. While on this diet, there were no significant alterations in the plasma lipid or lipoprotein values. Starting 1 day before the injection of the radioiodinated apoA-I and continuing throughout the experiment, the subjects were given 300 mg of potassium iodide three times a day.

Preparation of tracer apolipoproteins and lipoproteins

ApoA-I was isolated from normal controls and each of the hypoalpha patients as previously described (22). Purified apoA-I was iodinated with either ^{125}I or ^{131}I (NEZ 033L and NEZ 035H, NEN/DuPont, Boston, MA), in the presence of 6 M guanidine hydrochloride in 1 M glycine (pH 8.5), as previously described (23). Less than 0.5 mole of covalently bound iodine were present per mole of apoA-I, and the efficiency of iodination ranged from 15 to 45%. Iodinated apo-I was incubated with freshly isolated plasma (anticoagulated with 1 mg/ml of EDTA) from either control subjects or patients for 15 min at 37°C, adjusted to a density of 1.21 g/ml with solid KBr, and centrifuged for 24 h at 4°C in a 60 Ti rotor at 59,000 rpm in an L8-M ultracentrifuge (Beckman, Palo Alto, CA). The lipoprotein-containing supernatant was isolated by tube slicing, with more than 90% of the radioactive apoA-I being associated with these lipoproteins. The lipoproteins were dialyzed extensively in PBS, and human serum albumin was added at a final concentration of 1%, after which the lipoproteins were sterile filtered and tested for pyrogenicity and sterility. Doses of 50 μCi of ^{125}I-labeled apoA-I and 25 μCi of ^{131}I-labeled apoA-I were injected into each subject.

Experimental protocol

Each subject was injected with both ^{125}I- and ^{131}I-labeled apoA-I. After the injection, blood was drawn into tubes containing EDTA (1 mg/ml final concentration) at 10 min, at 3, 6, 12, 24, and 36 h, and on days 2, 3, 5, 7, 9, 11, and 14. Plasma was prepared by centrifugation at 1500 *g* for 30 min at 4°C. NaN_3 (0.05%) and aprotinin (1000 KIU/ml, Boehringer Mannheim GmbH, Germany) were added to the plasma. The radioactivity in 5-ml ali-

quots of the plasma was quantified in a Packard 5260 Autogamma counter (Packard Instrument Company, Downers Grove, IL).

Compositional analysis of plasma lipoproteins

Fasting plasma lipoprotiens were isolated by sequential ultracentrifugation at 4°C in a 40.3 rotor (Beckman, Palo Alto, CA) at the densities of 1.063 g/ml, 1.125 g/ml, and 1.210 g/ml (24). Total protein content was determined by the method of Lowry et al. (25), using BSA as a standard. Enzymic assays were utilized in the quantitations of total cholesterol (CII, cat. 276-64909, Wako Pure Chemical Industries, Ltd., TX), free cholesterol (cholesterol C, cat. 274-47109, Wako), phospholipids (phospholipids, cat. 996-54001, Wako) and triglycerides (triglycerides GPO-PAP, cat. 701 904, Boehringer Mannheim GmbH, Germany).

Analysis of apoA-I

Acid hydrolysis of apoA-I samples (0.5 nmol of protein) was performed for 24 h in 6 M HCL containing 2-mercaptoethanol (1:2000 v/v) at 108°C (22). Analyses were performed in a Beckman Model 6300 Amino Acid Analyzer equipped with a Hewlett-Packard Model 3390 A Integrator (Hewlett-Packard, Avondale, PA).

Two-dimensional gel electrophoresis (first dimension isoelectrofocusing at a pH range of 4–7, second dimension in 0.1% SDS, 15% acrylamide gel) was carried out on 3 μl of plasma and proteins were detected by silver staining as previously published (26).

Computer analysis of data

The residence times (RT) of the radiolabeled apoA-I in the kinetic studies were determined using the SAAM 29 program (27) on a VAX 11/780 computer (Digital Equipment Corp., Maynard, MA). The production rate of apoA-I was determined as $\frac{\text{apoA-I plasma pool (mg)}}{\text{RT (days)} \times \text{body weight (kg)}}$ where apoA-I plasma pool is the product of plasma apoA-I concentration times the plasma volume, with the plasma volume being assumed as 4% of body weight.

Other methods

Cholesterol and triglycerides were determined in plasma using the Gilford 3500 automated system analyzer. HDL cholesterol was quantitated after dextran sulfate precipitation of apoB-containing lipoproteins in plasma (28). A-I and A-II apolipoproteins were determined in plasma and HDL with a radial immunodiffusion assay as previously described (29). Statistical comparisons were made using the nonpaired Student's *t*-test.

RESULTS

The characteristics of the patients and control subjects are given in **Table 1.** All of the patients had decreased HDL cholesterol levels while apoA-I values were lower than average for the control population, but still within the normal range. Patients 1 and 3 were homozygous for the 3.3 kb PstI fragment of the apoA-I gene while patient 2 was heterozygous. Patients 1 and 2 were normotriglyceridemic at the time of the metabolic study, while patient 3 was hypertriglyceridemic. This patient was normotriglycleridemic at the time of ascertainment and met all of the criteria for familial hypoalpha. He was therefore included in the study.

Compositional studies were performed on HDL_2 and HDL_3 as isolated by sequential ultracentrifugation at the densities of 1.063–1.125 and 1.125–1.210 g/ml, respectively. On an absolute basis, hypoalpha patients had a low concentration of HDL_2 and normal levels of HDL_3 (**Table 2**). When the percent composition was determined for both subclasses, no marked abnormalities were noted. This absolute decrease in HDL_2, which is relatively cholesterol-enriched and protein-poor compared to HDL_3, is consistent with the results in Table 1, in which HDL cholesterol was decreased proportionately more than the apoA-I.

Two-dimensional gel electrophoresis was performed on plasma from the three patients and no abnormalities were noted in the apolipoprotein isoform patterns. **Fig. 1** illustrates the portion of the two-dimensional plasma gels con-

TABLE 1. Characteristics of patients and control subjects

Subject	Age	Sex	Weight	Cholesterol	Triglycerides	HDL Cholesterol	ApoA-I	PstI Fragment Length
	yr		*kg*		*mg/dl*			*kb*
Hypoalpha 1	29	M	69	156	203	28	100	3.3/3.3
Hypoalpha 2	32	M	70	169	126	35	96	3.3/2.2
Hypoalpha 3	40	M	73	240	437	26	109	3.3/3.3
Controls (n = 7)	19-24	6 M, 1 F	76 ± 11[a]	169 ± 19	76 ± 19	53 ± 14	126 ± 17	ND

[a]Mean ± SD; ND, not determined.

TABLE 2. Composition of HDL_2 and HDL_3

Subject	HDL_2				HDL_3			
	Protein	Cholesterol	Triglyceride	Phospholipid	Protein	Cholesterol	Triglyceride	Phospholipid
	mg/ml (%)				*mg/ml (%)*			
Hypoalpha 1	0.19 (53)	0.06 (17)	0.01 (3)	0.10 (28)	0.69 (66)	0.12 (11)	0.03 (3)	0.21 (20)
Hypoalpha 2	0.27 (49)	0.10 (18)	0.02 (4)	0.16 (26)	0.49 (58)	0.13 (15)	0.02 (2)	0.21 (25)
Hypoalpha 3	0.12 (52)	0.04 (17)	0.01 (4)	0.06 (26)	0.91 (71)	0.12 (9)	0.05 (4)	0.20 (16)
Control (n = 4)	0.36 ± 0.06[a](47)	0.16 ± 0.02 (21)	0.01 ± 0.01 (1)	0.24 ± 0.04 (31)	0.56 ± 0.04[a] (62)	0.12 ± 0.02 (13)	0.03 ± 0.02 (3)	0.20 ± 0.01 (22)

[a]Mean ± SD.

taining apoA-I. When compared with apoA-I from normal controls, apoA-I from the hypoalpha patients had no shift in charge or molecular weight. The composition of apoA-I isolated from all three hypoalpha subjects was indistinguishable from normal apoA-I by amino acid analysis (**Table 3**). Utilizing these methods, no structural abnormalities were detected in the apoA-I isolated from the hypoalpha patients.

To better define the metabolic abnormality in the hypoalpha patients, the kinetics of apoA-I metabolism were determined. Normal apoA-I and apoA-I isolated from each of the hypoalpha probands were radiolabeled and injected simultaneously into normal control subjects. There was virtually no difference between the rate of catabolism of normal and hypoalpha apoA-I in the normal subjects studies (**Table 4** and **Fig. 2**). There was also no significant difference in the rate of catabolism of normal apoA-I and hypoalpha apoA-I in the hypoalpha study subjects (Table 4 and **Fig. 3**). These studies establish that the apoA-I isolated from hypoalpha patients was metabolically normal.

In contrast, there was a significant difference in the kinetic parameters of autologous apoA-I metabolism in hypoalpha and normal control subjects. The hypoalpha subjects had a decreased plasma residence time, i.e., an increased fractional catabolic rate, for apoA-I compared to the normal controls (**Table 5** and **Fig. 4**). The apoA-I production rate, on the contrary, was the same in the

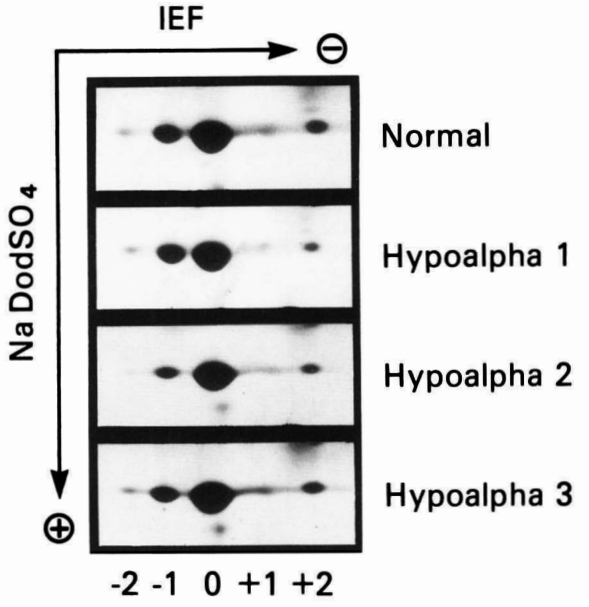

Fig. 1. Comparison of the isoforms of normal apoA-I and hypoalpha apoA-I by two-dimensional gel electrophoresis. Isoelectric focusing was performed on 3 μl of plasma in the horizontal direction followed by $NaDodSO_4$ gel electrophoresis in the descending direction. Proteins were detected by silver stain. Only the portion of the two-dimensional gel containing the apoA-I isoforms is shown to simplify the figure. Panels are, from the top, normal apoA-I and hypoalpha apoA-I from subjects 1–3, respectively.

TABLE 3. Amino acid composition of hypoalpha apoA-I

Amino Acid	Normal[a]	Hypoalpha 1	Hypoalpha 2	Hypoalpha 3	Theoretical
Asp	21.2 ± 0.6	20.8	21.2	20.7	21
Thr	10.0 ± 0.3	10.0	9.9	10.0	10
Ser	14.9 ± 0.4	14.9	15.1	14.7	15
Glu	46.8 ± 1.4	46.0	46.2	45.2	46
Pro	10.6 ± 0.3	10.5	10.2	10.5	10
Gly	10.8 ± 0.3	10.3	12.5	10.2	10
Ala	19.6 ± 0.6	19.3	19.4	19.2	19
Cys					0
Val	11.8 ± 0.4	11.9	12.1	12.0	13
Met	2.9 ± 0.1	2.2	2.6	2.8	3
Ileu					0
Leu	36.9 ± 1.1	37.2	37.2	35.8	37
Tyr	7.0 ± 0.2	7.1	7.0	7.0	7
Phe	6.0 ± 0.2	6.2	6.3	6.3	6
His	4.7 ± 0.1	4.6	4.9	4.7	5
Lys	20.2 ± 0.6	20.4	20.7	20.6	21
Arg	16.7 ± 0.5	15.3	15.9	16.9	16

[a]All data are from 24 h hydrolysis.

hypoalpha and normal control subjects (Table 5). These combined results indicate that the decreased plasma apoA-I concentration in the subjects with familial hypoalpha is due entirely to a decreased plasma residence time of a structurally and metabolically normal apoA-I.

DISCUSSION

Primary familial hypoalphalipoproteinemia is characterized by low levels of HDL cholesterol with normal total plasma cholesterol and triglyceride levels (12, 13). A 3.3 kb restriction endonuclease fragment, generated near the 3' end of the apoA-I gene during digestion with PstI, has been reported to consegregate with hypoalphalipoproteinemia in a family with this syndrome (20) and to occur with an increased prevalence in the probands of 12 kindreds with familial hypoalpha when compared with a control population (21). In addition, it is clear that the base substitution resulting in the PstI polymorphism is not the causative mutation since the majority of individuals in a nonselected population with this polymorphism have normal HDL and apoA-I levels (21). The location of the polymorphic site near the apoA-I gene suggests that the 3.3 kb apoA-I fragment is in linkage disequilibrium with a mutation in the apoA-I gene which

TABLE 4. Residence times of normal and hypoalpha apoA-I in normal and hypoalpha subjects

Subject	Residence Time (days)	
	Normal ApoA-I	Hypoalpha ApoA-I
Hypoalpha 1[a]	3.58 ± 0.05[b]	3.94 ± 0.06
Hypoalpha 2	3.83 ± 0.09	3.87 ± 0.02
Hypoalpha 3	3.54 ± 0.02	4.28 ± 0.03
Mean ± SD	3.65 ± 0.16[c]	4.03 ± 0.22[d]
Control 1	4.97 ± 0.07	5.36 ± 0.08
Control 2	4.89 ± 0.07	5.21 ± 0.07
Control 3	5.46 ± 0.12	5.62 ± 0.12
Control 4	5.44 ± 0.06	5.79 ± 0.06
Control 5	4.70 ± 0.04	4.91 ± 0.04
Control 6	4.28 ± 0.04	4.42 ± 0.04
Control 7	4.63 ± 0.08	5.51 ± 0.17
Mean ± SD	4.91 ± 0.43	5.26 ± 0.47

[a]Controls 1 and 2 were studied with hypoalpha 1, control 3 with hypoalpha 2, and controls 4–7 with hypoalpha 3.
[b]Mean ± SD; this is an estimate of the fit of the computer-generated multiexponential decay curve to the actual data points (27).
[c]$P < 0.005$ when compared to normal apoA-I in control subjects.
[d]$P < 0.005$ when compared to hypoalpha apoA-I in control subjects; $P < 0.02$ when compared to normal apoA-I in control subjects.

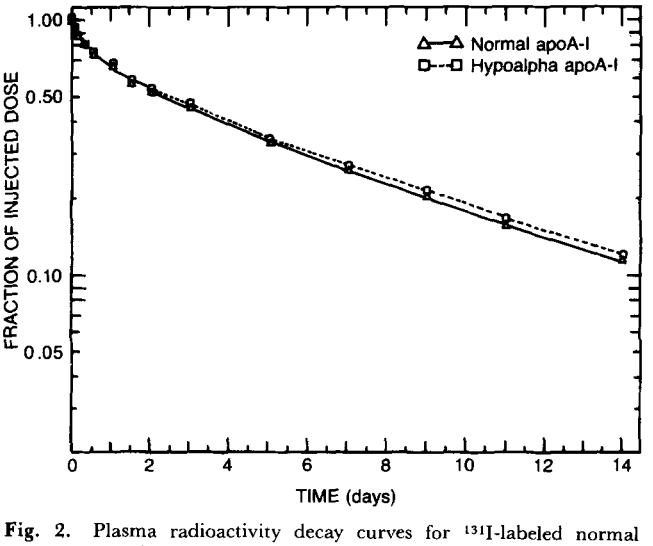

Fig. 2. Plasma radioactivity decay curves for ^{131}I-labeled normal apoA-I (△—△) and ^{125}I-labeled hypoalpha apoA-I (□---□) in a normal subject.

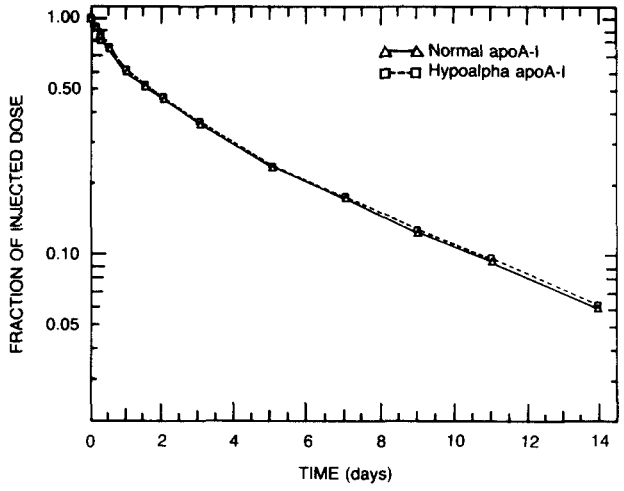

Fig. 3. Plasma radioactivity decay curves for ^{131}I-labeled normal apoA-I (△ — △) and ^{125}I-labeled hypoalpha apoA-I (□---□) in a hypoalpha subject (hypoalpha 2).

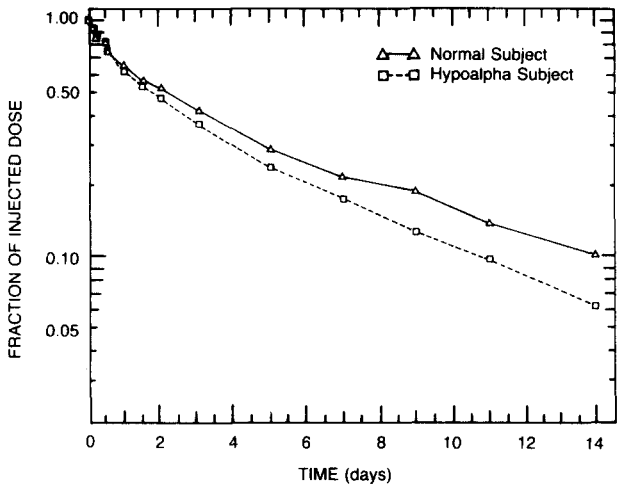

Fig. 4. Plasma radioactivity decay curves for ^{131}I-labeled normal apoA-I in a normal subject (△ — △) and ^{125}I-labeled hypoalpha apoA-I in a hypoalpha subject (hypoalpha 2) (□---□).

would be the causative defect in familial hypoalpha. A mutation in the regulatory portion of the apoA-I gene could result in a decreased transcriptional rate for the apoA-I mRNA, while an alteration in the coding portion of the gene could lead to either a defect in the translation and secretion of apoA-I or an increased catabolic rate of the secreted protein.

When comparing the composition of HDL from the study subjects with the PstI polymorphism and hypoalpha with the HDL from the normal controls, the most striking difference was a consistent decrease in the quantity of each of the components of HDL_2, while the concentration of HDL_3 was normal. The percent composition of both HDL_2 and HDL_3 from the three study subjects was normal, and therefore, the decreased HDL_2 levels were due to a decreased number of particles with normal composition. Since HDL_2 is relatively enriched in cholesterol and depleted in proteins compared to HDL_3, this results in a proportionately greater decrease in HDL cholesterol than apoA-I. In addition to the normal percent composition of HDL, the amino acid analysis and two-dimensional gel electrophoretic pattern of the apoA-I from the hypoalpha subjects were also normal. Therefore, there was no evidence for a structural abnormality in either HDL or apoA-I from the subjects with familial hypoalpha and the 3.3 kb PstI polymorphism.

In order to determine whether the apoA-I from the study subjects was metabolically normal, hypoalpha and normal apoA-I were labeled with radioactive iodine and injected simultaneously into the same subject. Utilizing this experimental design, there is only a single variable and one can directly determine whether the apoA-I from the two different types of subjects are metabolically different. When the rates of catabolism of hypoalpha and normal apoA-I were compared in the same subject, either in normal controls or hypoalpha subjects, there was virtually no difference in the residence times for the two forms of apoA-I. This indicates that the hypoalpha apoA-I is metabolically normal. Since there were no abnormalities in the composition or two-dimensional gel pattern of the apoA-I from the hypoalpha subjects, and their apoA-I was metabolically normal, we conclude that the apoA-I protein in these subjects is normal and, by inference, that the coding portion of the apoA-I gene is normal.

TABLE 5. Kinetic parameters of apoA-I metabolism in patients with familial hypoalphalipoproteinemia and control subjects

Subject	ApoA-I	Residence Time	Fractional Catabolic Rate	Production Rate
	mg/dl	*days*	*day^{-1}*	*mg/kg · day*
Hypoalpha 1	100	3.94	0.25	10.16
Hypoalpha 2	96	3.87	0.26	9.91
Hypoalpha 3	109	4.28	0.23	10.20
Control (n = 7)	126 ± 17[a]	4.91 ± 0.43	0.20 ± 0.02	10.28 ± 1.23

[a]Mean ± SD.

Differences were readily demonstrated, though, in the kinetic parameters of apoA-I metabolism. The hypoalpha subjects had a decreased residence time, i.e., an increased fractional rate of metabolism, for both normal and autologous apoA-I when compared to normal apoA-I in normal subjects, while their production rates of apoA-I were normal. Therefore, the decreased plasma apoA-I levels in these subjects were due to an increased rate of catabolism. Since the apoA-I production rates were normal in the hypoalpha subjects, the low apoA-I and HDL levels cannot be due to a decrease in the rate of apoA-I transcription, translation, or secretion. These studies indicate that the rates of apoA-I biosynthesis, and by inference the regulatory elements of the apoA-I gene, are normal in these subjects. With the strong evidence that both the regulatory and the coding portions of the apoA-I gene are normal, we conclude that the apoA-I gene is normal in these subjecjts and that the PstI polymorphism linked to the apoA-I gene is not a marker for a mutation in apoA-I.

It is of interest that patients 1 and 3 were homozygous for the PstI RFLP while Patient 2 was heterozygous, even though all three of these patients had very similar kinetic parameters of apoA-I metabolism and could not be differentiated on the basis of the kinetic results. There are two possible explanations for this; the first being that the mutation resulting in hypoalpha is dominant, and therefore heterozygous and homozygous subjects are phenotypically the same. The second is that even though patients 1 and 3 are homozygous for the PstI polymorphism, they are heterozygous for the mutation that results in hypoalpha in that there is not a perfect association between the PstI RFLP and familial hypoalpha. One will have to await the identification of the actual causative mutation for hypoalphalipoproteinemia in these subjects before these two possibilities can be differentiated.

The results of these studies are most consistent with the PstI polymorphism being a marker for an abnormality in one of the other genes in the multigene complex on chromosome 11 of which the apoA-I gene is a part. The apoA-IV or apoC-III genes are possible candidate genes for the mutation since they are both involved in lipoprotein metabolism and are in this multigene complex. We were unable to detect, however, any structural abnormalities in the protein products of these two genes by two-dimensional gel electrophoresis of whole plasma (data not shown), although, of course, this does not eliminate the possibility of a functional abnormality in either of these proteins that is not detected on two-dimensional gels. On the other hand, the primary mutation may be in an as yet unidentified, but closely linked, gene that is important in the regulation of HDL and apoA-I catabolism.

Schaefer et al. (23) investigated the kinetics of apoA-I metabolism in three normotriglyceridemic subjects who had similarly decreased HDL cholesterol and apoA-I levels as did our subjects. These individuals also had hypercatabolism as the kinetic etiology of hypoalphalipoproteinemia as did the subjects studied by Ghiselli, Beigel, and Gotto (30). In contrast, Le and Ginsberg (31) found reduced apoA-I production rates as the kinetic etiology of the decreased apoA-I levels in their hypoalphalipoproteinemic individuals. These combined results, with the inclusion of our studies, indicate a metabolic and certain genetic heterogeneity in hypoalphalipoproteinemic humans.

In conclusion, the results of the present studies on the structure and metabolism of apoA-I from three subjects with familial hypoalphalipoproteinemia and a 3.3 kb PstI polymorphism linked to the apoA-I gene strongly indicate that the apoA-I gene is normal in these hypoalphalipoproteinemic subjects. Additional studies will be needed to determine the exact location of the molecular defect in this disease. The elucidation of this mutation will provide important new insights into the regulation of HDL and apoA-I catabolism in humans.

We would like to thank Mrs. Patti Riggs and the dietary staff of the NIH Clinical Center for assistance in the dietary portion of the studies, the nursing staff on ward 8E for excellent care of the study subjects, Ms. Joanie Gault for preparation of the manuscript, and Mrs. Santi Datta, Barbara Winterrowd, Diane Wilson, and Marie Kindt for technical assistance. In addition, we would like to express our appreciation to the normal volunteers and the hypoalphalipoproteinemic subjects who made this study possible.

Manuscript received 7 March 1989 and in revised form 18 June 1990.

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