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In vivo metabolism of a mutant apolipoprotein, apoA-I_{Iowa}, associated with hypoalphalipoproteinemia and hereditary systemic amyloidosis

Daniel J. Rader,[1] Richard E. Gregg,[2] Martha S. Meng, Juergen R. Schaefer,[3] Loren A. Zech, Merrill D. Benson,* and H. Bryan Brewer, Jr.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, and Departments of Medicine and Medical Genetics,* Indiana University School of Medicine, Indianapolis, IN 46202

Abstract Apolipoprotein (apo) A-I is the major protein constituent of plasma high density lipoproteins (HDL). A kindred has been identified in which a glycine to arginine mutation at residue 26 in apoA-I is associated with hypoalphalipoproteinemia and hereditary systemic amyloidosis. We isolated the mutant protein, termed apoA-I_{Iowa}, from the plasma of an affected subject and studied its in vivo metabolism compared to that of normal apoA-I in two heterozygous apoA-I_{Iowa} subjects and two normal controls. Normal and mutant apoA-I were radioiodinated with ^{131}I and ^{125}I, respectively, reassociated with autologous plasma lipoproteins, and simultaneously injected into all subjects. Kinetic analysis of the plasma radioactivity curves demonstrated that the mutant apoA-I_{Iowa} was rapidly cleared from plasma (mean fractional catabolic rate [FCR] 0.559 day^{-1}) compared with normal apoA-I (mean FCR 0.244 day^{-1}) in all four subjects. The FCR of normal apoA-I was also substantially faster in the heterozygous apoA-I_{Iowa} subjects (mean FCR 0.281 $days^{-1}$) than in the normal controls (mean FCR 0.203 $days^{-1}$). Despite the rapid removal from plasma of apoA-I_{Iowa}, the cumulative urinary excretion of its associated radioactivity after 2 weeks (44%) of the injected dose) was substantially less than that associated with normal apoA-I (78% of injected dose), indicating extravascular sequestration of radiolabeled apoA-I_{Iowa}. Therefore, the single amino acid substitution in apoA-I_{Iowa} results in both rapid clearance of apoA-I, explaining the low levels of plasma HDL and apoA-I, as well as extravascular sequestration of the mutant apolipoprotein, consistent with the formation of amyloid deposits containing aggregates of apoA-I_{Iowa}, in heterozygous carriers of this unique apoA-I variant.—**Rader, D. J., R. E. Gregg, M. S. Meng, J. R. Schaefer, L. A. Zech, M. D. Benson, and H. B. Brewer, Jr.** In vivo metabolism of a mutant apolipoprotein, apoA-I_{Iowa}, associated with hypoalphalipoproteinemia and hereditary systemic amyloidosis. *J. Lipid Res.* 1992. **33:** 755-763.

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Apolipoprotein (apo) A-I is the major protein constituent of plasma high density lipoproteins (HDL). A single polypeptide of 243 amino acids, it has a molecular mass of approximately 28,000 daltons and contains no carbohydrate. ApoA-I serves as a cofactor for the activation of the plasma enzyme LCAT (1), promotes cholesterol efflux from cells (2), and may act as a ligand for a putative HDL receptor (3). Decreased plasma levels of HDL-cholesterol (4) and apoA-I (5) are epidemiologically associated with an increased risk of atherosclerotic cardiovascular disease.

Several variant forms of apoA-I have been detected in plasma (6-13), but only four are associated with low levels of HDL and none with premature atherosclerosis or other diseases. A new variant of apoA-I was recently reported in a kindred with hereditary systemic amyloidosis. Iowa-type hereditary systemic amyloidosis, also known as familial amyloidotic polyneuropathy type III, was first described by Van Allen, Frohlich, and Davis in 1969 (14). In this large kindred, the onset of symptoms occurs in the third or fourth decade and the disease is characterized by lower extremity peripheral neuropathy, nephropathy, and peptic ulcer disease. Transmission occurs in an autosomal dominant fashion. Nichols et al. (15) reported that amyloid fibrils isolated at autopsy from a member of this kindred consisted primarily of a protein identical to the amino-terminal 83 residues of mature apoA-I, with the exception of a substitution of arginine for glycine at residue 26. Serum from the proband was shown by isoelectric focusing to contain both normal apoA-I as well as a variant form of apoA-I with a + 1 charge shift, consistent with an arginine for glycine substitution. Direct sequencing of genomic DNA from the proband confirmed

Abbreviations: FCR, fractional catabolic rate; HDL, high density lipoprotein; RT, residence time; PR, production rate.

[1]To whom correspondence should be addressed at: Bldg. 10, Room 7N117, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

[2]Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

[3]Present address: Philipps University, Marburg, Germany.

the expected point mutation in codon 26 of the apoA-I gene (16), and three other affected individuals were also determined to have the same mutation. With allele-specific amplification by the polymerase chain reaction, complete concordance between the mutation and the presence of the variant apoA-I, termed apoA-I_{Iowa}, was demonstrated in the plasma of several family members.

In this kindred, heterozygotes for the mutant apoA-I_{Iowa} also have HDL cholesterol levels below the tenth percentile. We hypothesized that the mutant apoA-I_{Iowa} is rapidly catabolized compared with normal apoA-I, accounting for the low levels of HDL, and that it is sequestered in extravascular tissues, resulting in amyloid formation. In order to gain further insight into the pathophysiology of the hypoalphalipoproteinemia and hereditary systemic amyloidosis found in this kindred, we investigated the in vivo metabolism of apoA-I_{Iowa} and normal apoA-I in both apoA-I_{Iowa} and normal subjects.

METHODS

Study subjects

The subjects studied included two asymptomatic heterozygotes for the apoA-I_{Iowa} mutation and two normolipidemic control subjects. The apoA-I_{Iowa} subjects were sisters who were both demonstrated to be heterozygotes for the mutation by two-dimensional gel electrophoresis of whole plasma **(Fig. 1)**, isoelectric focusing of plasma apoA-I followed by immunoblotting with anti-apoA-I antibodies, and by direct DNA analysis as previously described (16). Clinical, lipid and apolipoprotein data on all study subjects are presented in **Table 1.** Values are the mean of five fasting determinations made while the subjects were on the metabolic diet. Both of the apoA-I_{Iowa} subjects had HDL cholesterol levels below the tenth percentile for their age and sex (17). Body weights and plasma lipid and apolipoprotein levels remained in steady state in all four subjects throughout the course of the study. All subjects had normal fasting glucose levels and normal thyroid, liver, and kidney function. Clinical evaluation, including abdominal fat pad aspiration for Congo red staining material, revealed no evidence of amyloidosis in the apoA-I_{Iowa} subjects. The subjects were on no medications during the study. All subjects gave informed consent and the study protocol was approved by the Clinical Research Subpanel of the National Heart, Lung, and Blood Institute.

TABLE 1. Characterization of study subjects

Subject		Sex	Age	BMI	TG	TC	HDL-C	ApoA-I	ApoA-II
			yr	*kg/m²*			*mg/dl*		
ApoA-I_{Iowa}	1	F	31	22.5	113	176	27	69	24
ApoA-I_{Iowa}	2	F	28	31.2	61	190	32	73	26
Control	1	F	20	20.2	104	185	47	119	33
Control	2	M	20	22.7	87	213	57	133	30

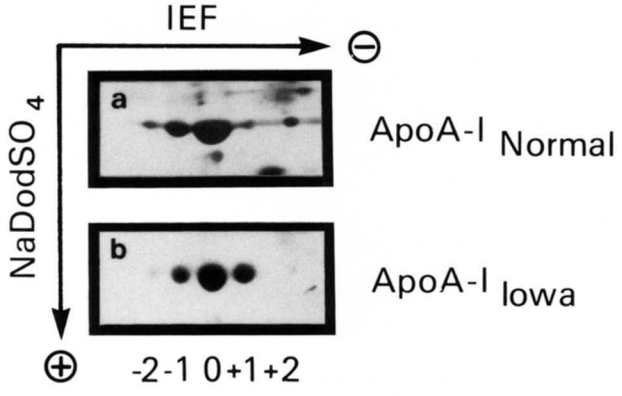

Fig. 1. Representative two-dimensional gel electrophoretograms of plasma from a normal subject and from a subject heterozygous for the apoA-I_{Iowa} mutation. Whole plasma from each subject was separated in the horizontal direction by isoelectric focusing and in the vertical direction by $NaDodSo_4$ gel electrophoresis. Only the apoA-I region is shown. In comparison to normal, the plasma from the apoA-I_{Iowa} subject has a large amount of protein in the +1 position, representing the variant apoA-I_{Iowa}.

Isolation of normal apoA-I and apoA-I_{Iowa}

ApoA-I was isolated from the HDL of a healthy donor with normal lipid and apolipoprotein levels using gel permation chromatography as previously described (18). ApoA-I_{Iowa} was purified from the HDL from one of the subjects heterozygous for the mutation. HDL were isolated by preparative ultracentrifugation (d 1.063–1.21 g/ml), dialyzed against 0.01 M NH_4HCO_3, lyophilized, and delipidated with chloroform–methanol 2:1. Samples were solubilized in 10 M urea, 5% mercaptoethanol, and 2% Servalyte (Serva, Heidelberg, Germany). Preparative isoelectric focusing was carried out in a vertical slab gel apparatus (Bio-Rad, Richmond, CA) using a 3-mm thick gel with a pH gradient from 5 to 7. Gels were run at 200 volts for 20 h at 4°C. After electrophoresis, one lane of the gel was stained with Coomassie blue G to visualize the bands. The band in the +1 position, corresponding to the apoA-I_{Iowa} protein, was cut from the unstained portion of the gel. The apoA-I_{Iowa} protein was recovered by electrophoretic elution into a dialysis bag containing 0.01 M NH_4HCO_3 and 1% $NaDodSO_4$ for 18 h at 4°C. The isolated protein was dialyzed against 0.01 M NH_4HCO_3, lyophylized, and desalted on a Sephadex G100 (0.9 × 94 cm, Pharmacia) column equilibrated with 0.01 M NH_4HCO_3. Purified normal apoA-I and apoA-I_{Iowa} were analyzed for purity by $NaDodSO_4$ gel electrophoresis and isoelectric focusing.

Iodination of apoA-I

Studies were performed using purified radiolabeled apolipoproteins. Previous studies have demonstrated that

this approach gives results similar to those obtained by iodination of intact HDL (19, 20). Purified apolipoproteins were iodinated by a modification of the iodine monochloride method as previously described (21). Briefly, lyophylized normal apoA-I and apoA-I_{Iowa} were dissolved in a 6 M guanidine-HCI, 1 M glycine (pH 8.5) buffer. Five mCi of $Na^{131}I$ (normal apoA-I) or $Na^{125}I$ (apoA-I_{Iowa}) were added, followed by iodine monochloride. Approximately 0.5 mole iodine was incorporated per mole protein. ^{131}I-apoA-I and ^{125}I-apoA-I_{Iowa} were reassociated with separate aliquots of fresh fasting autologous plasma by incubation at 37°C for 15 min. Plasma was adjusted to 1.21 g/ml with solid KBr, ultracentrifuged at 59K rpm for 24 h at 4°C in a 60 Ti rotor with an L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA), and plasma lipoproteins (d < 1.21 g/ml) were obtained by tube slicing. Samples were dialyzed extensively against sterile PBS containing 0.01% EDTA. Human serum albumin was added to a final concentration of 10 mg/ml. Samples were passed through a 0.22 μm Millipore filter and tested for pyrogenicity and sterility. All samples were pyrogen-free, sterile, and contained no free iodine as judged by radiochromatography. Radiolabeled normal apoA-I and apoA-I_{Iowa} migrated to the expected positions on both $NaDodSO_4$ gel electrophoresis and isoelectric focusing.

Study protocol

Three days before the start of the study the subjects were placed on an isoweight diet containing 42% of calories as fat (polyunsaturated to saturated fat ratio 0.2), 42% carbohydrate, and 16% protein, with 200 mg of cholesterol per 1000 kcal. Meals were given three times per day and the diet was continued for the duration of the study. One day prior to the start of the study the subjects were given a potassium iodide solution (900 mg per day in divided doses) which was continued for the duration of the study.

Subjects were injected intravenously after a 12-h fast with 10 μCi of ^{131}I-radiolabeled normal apoA-I and 30–50 μCi of ^{125}I-radiolabeled apoA-I_{Iowa}. Blood samples were obtained at 10 min, 1, 3, 6, 9, 12, 16, 24, and 36 h, daily through day 6, and on days 8, 10, and 13. Urine was collected continuously throughout the study, with new collections beginning every 4 h for the first day and then daily thereafter.

Blood samples were drawn into tubes containing EDTA at a final concentration of 0.1%. Plasma was separated by low speed centrifugation (2300 rpm, 30 min) at 4°C. Sodium azide and aprotinin were added to plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivity in plasma and urine was quantitated in a Packard Autogamma 5260 gamma counter (Packard Instrument Co., Morton Grove, IL).

Ultracentrifugation procedures

HDL_2, HDL_3, and the d > 1.21 g/ml fraction were isolated from 5 ml plasma by sequential ultracentrifugation as previously described (22). Radioactivity in the top and bottom fractions after each centrifugation was determined, and the ratio of the radioactivity in the top/(top + bottom) was used to determine the total radioactivity in each density fraction at each time point.

Analysis of kinetic data

Plasma decay curves were constructed by dividing the plasma radioactivity at each time point by the radioactivity in the 10-min plasma sample. Multiexponential functions were fit to the plasma decay curves using the SAAM30 program (23). The residence times (RT) were obtained from the areas under the plasma decay curves. The fractional catabolic rates (FCR) are the reciprocals of the residence times. Apolipoprotein production rates (PR) were calculated using the formula: production rate = (plasma apolipoprotein concentration) × (plasma volume) × (fractional catabolic rate)/(body weight). Plasma volume was assumed to be 4% of total body weight.

Other analytical methods

Plasma cholesterol and triglycerides were quantitated by automated enzyme techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol was determined in plasma after dextran sulfate precipitation (24). Plasma apoA-I was quantitated by

TABLE 2. Percent distribution of radiolabeled apoA-I among HDL density subfractions 10 min after injection

		ApoA-I_{normal}			ApoA-I_{Iowa}		
Subject		HDL_2	HDL_3	d>1.21[a]	HDL_2	HDL_3	d>1.21[a]
ApoA-I_{Iowa}	1	32.7	60.1	6.2	23.1	47.2	25.8
ApoA-I_{Iowa}	2	24.5	66.8	7.8	21.7	50.4	24.5
Control	1	43.8	49.3	5.1	33.5	39.3	23.5
Control	2	37.1	52.4	7.6	30.7	41.1	25.1

Values do not add up to 100% because of small amounts of tracer in VLDL and LDL.
[a]d>1.21 refers to the fraction of density > 1.21 g/ml.

ELISA (25). There was no evidence of altered reactivity of apoA-I_{Iowa} compared with normal apoA-I in this assay. The ratio of apoA-I_{Iowa} to normal apoA-I in the plasma of the apoA-I_{Iowa} subjects was determined by scanning densitometric analysis of two-dimensional electrophoretograms of plasma from apoA-I_{Iowa} subjects. The apoA-I_{Iowa} plasma level was determined by multiplying the fraction apoA-I_{Iowa}/apoA-I_{Iowa} + normal apoA-I) by the total plasma apoA-I concentration.

RESULTS

The distribution of radiolabeled normal apoA-I and apoA-I_{Iowa} in the plasma 10 min after injection is shown in **Table 2.** In all four subjects, there was less radiolabeled apoA-I_{Iowa} in HDL_2 and HDL_3 and more in the d > 1.21 g/ml fraction compared with normal apoA-I. The distributions of both radiolabeled apoA-I_{Iowa} and normal apoA-I were shifted from HDL_2 to HDL_3 in the apoA-I_{Iowa} heterozygotes compared with the control subjects.

The plasma curves of radiolabeled apoA-I_{Iowa} and normal apoA-I in the two apoA-I_{Iowa} subjects are shown in **Fig. 2A**. The turnover of apoA-I_{Iowa} was markedly faster than that of normal apoA-I. Fig. 2B depicts the cumulative urinary excretion of radioactivity derived from degraded protein in the two apoA-I_{Iowa} subjects. For the first 3 days there was more rapid excretion of radioactivity derived from apoA-I_{Iowa}. However, by the end of 2 weeks there was significantly less cumulative urinary excretion of the radioactivity associated with the apoA-I_{Iowa} protein than of that associated with normal apoA-I, despite the more rapid clearance of the mutant apoA-I_{Iowa} from plasma. Therefore, a substantial portion of the radioactivity associated with apoA-I_{Iowa} was removed from the plasma but did not appear in the urine within the 2 week duration of the study. This is illustrated by the data presented in Fig. 2C. At each time point, one minus the cumulative fraction of injected dose excreted in the urine equals the fraction of injected dose which remains in the body. This value minus the fraction of injected dose remaining in the plasma then represents the radioactivity, as a fraction of injected dose, which has entered an extravascular compartment. By the end of 2 weeks there was a substantial difference between the mutant and normal A-I apolipoproteins, with 56% of the apoA-I_{Iowa} but only 22% of the normal apoA-I remaining in non-plasma compartments. Furthermore, the sequestered apoA-I_{Iowa} was resistant to degradation and removal from its extravascular sites, whereas the normal apoA-I continued to be removed from the non-plasma compartments and degraded.

In order to establish that this aberrant metabolism is an intrinsic property of the apoA-I_{Iowa} protein, the same radiolabeled A-I apolipoproteins were studied in two normal control subjects. **Fig. 3A and 3B** illustrate the plasma curves and urinary accumulation of radioactivity in these two normal subjects. As was observed in the apoA-I_{Iowa} subjects, the apoA-I_{Iowa} protein was catabolized at a much faster rate than normal apoA-I and the total cumulative urinary excretion of apoA-I_{Iowa}-associated radioactivity by the end of 2 weeks was substantially less than that of normal apoA-I. Thus, apoA-I_{Iowa} appears to accumulate in extravascular compartments in normal (Fig. 3C) as well as in apoA-I_{Iowa} subjects.

The kinetic parameters of apoA-I_{Iowa} and normal apoA-I are presented in **Table 3**. The FCRs of apoA-I_{Iowa}

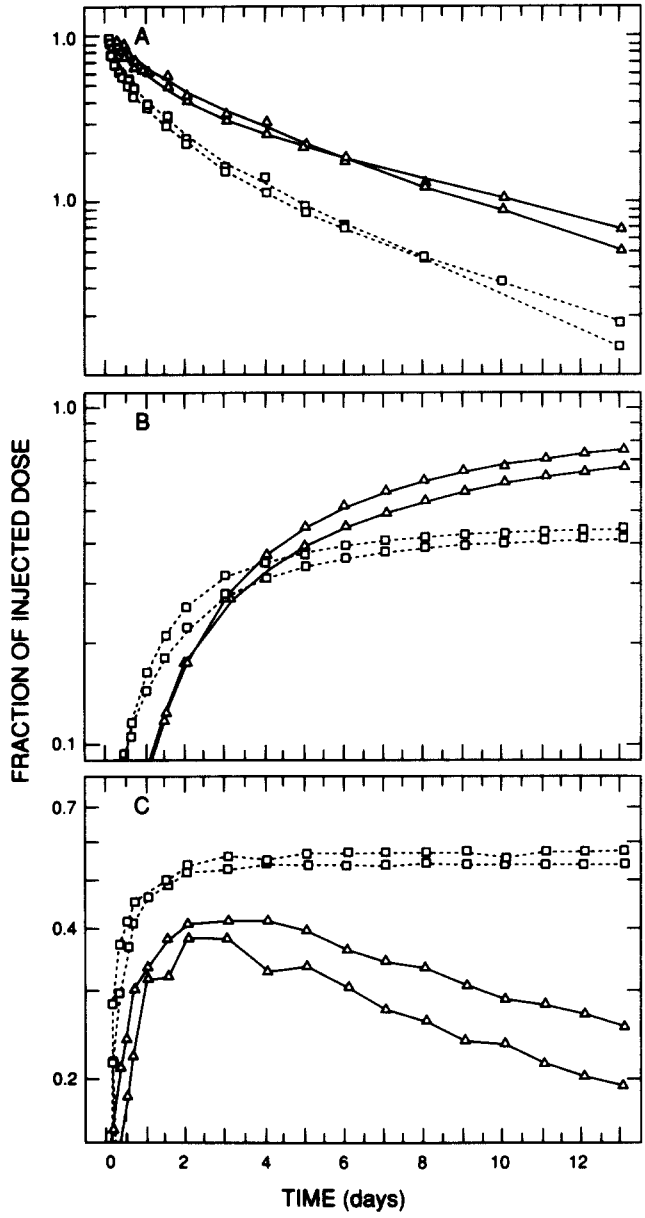

Fig. 2. Metabolism of apoA-I_{Iowa} (squares and dotted lines) and normal apoA-I (triangles and solid lines) in two apoA-I_{Iowa} subjects. A) Whole plasma decay curves; B) cumulative urinary excretion; C) extravascular accumulation in the body.

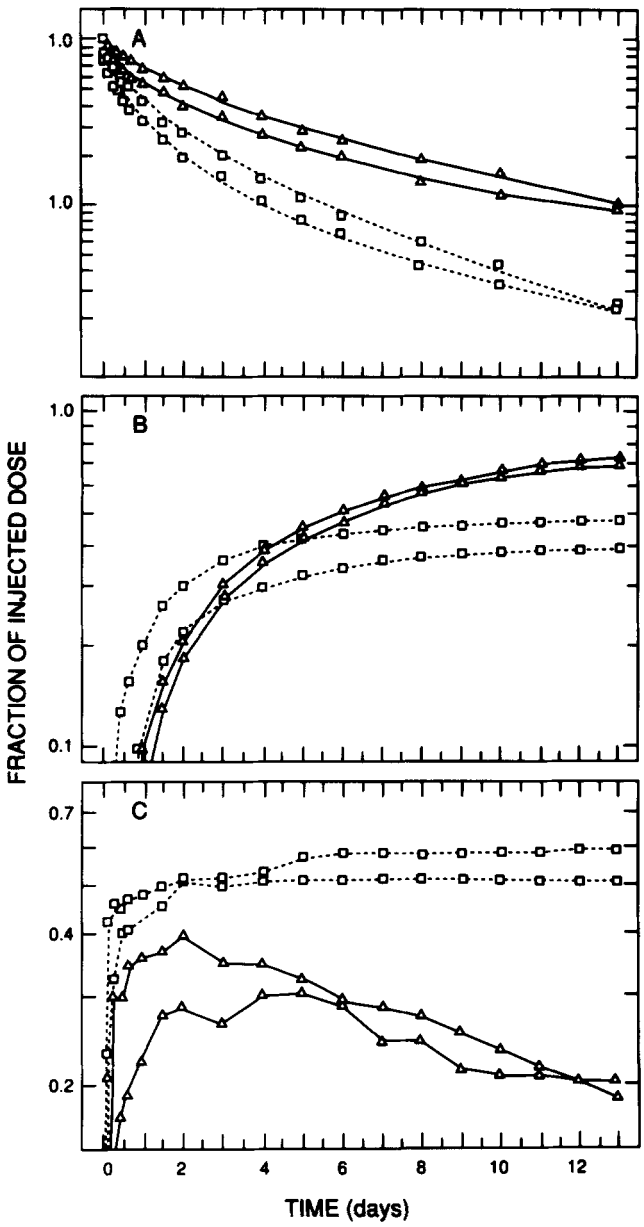

Fig. 3. Metabolism of apoA-I_{Iowa} (squares and dotted lines) and normal apoA-I Triangles and solid lines) in two normal subjects. A) Whole plasma decay curves; B) cumulative urinary excretion; C) extravascular accumulation in the body.

were more than twice those of normal apoA-I in all four subjects. The plasma concentration of apoA-I_{Iowa} is substantially lower than that of normal apoA-I in the affected subjects. The production rates of apoA-I_{Iowa} and normal apoA-I are similar, indicating that the lower concentration of apoA-I_{Iowa} is due solely to its increased catabolic rate and not to a decreased production rate. The apoA-I_{Iowa} subjects catabolized normal apoA-I substantially faster (mean FCR 0.281 $days^{-1}$) than did the two normal subjects (mean FCR 0.208 $days^{-1}$) and the historical controls (mean FCR 0.203 ± 0.016 $days^{-1}$). The total apoA-I production rates in the apoA-I_{Iowa} subjects were similar to those in the normal subjects. Therefore, the decreased total plasma apoA-I concentration in apoA-I_{Iowa} heterozygotes is due to increased fractional catabolic rates of both normal and mutant apoA-I.

The more rapid catabolism of apoA-I_{Iowa} than normal apoA-I from plasma was not due solely to differential extravascular distribution, as illustrated by the representative urine/plasma (U/P) radioactivity ratio curves in **Fig. 4**. In both the apoA-I_{Iowa} as well as the normal subjects, the apoA-I_{Iowa} U/P ratios were substantially higher and more heterogeneous than those for normal apoA-I. They peaked at day 3, then decreased and remained parallel, but a higher level, than those of normal apoA-I for the remainder of the study.

The turnover of apoA-I_{Iowa} and normal apoA-I in the HDL density subfractions is illustrated in **Fig. 5**. The difference in turnover rates between apoA-I_{Iowa} and normal apoA-I was least pronounced in HDL_2, greater in HDL_3, and most marked in the d > 1.21 g/ml fraction. These differences between apoA-I_{Iowa} and normal apoA-I among HDL subfractions were seen in both the apoA-I_{Iowa} (Fig. 5A) and the normal (Fig. 5B) subjects.

DISCUSSION

ApoA-I is essential for the formation of normal HDL. Inability to synthesize apoA-I results in marked HDL deficiency and premature coronary artery disease (26–28). Although a number of variant forms of apoA-I have been identified, only four have been shown to be associated with decreased plasma HDL concentrations. ApoA-$I_{pro165\ to\ arg}$ is associated in heterozygotes with mean HDL levels that are 58% those of unaffected family members (9). Heterozygotes for apoA-$I_{202:fs}$ have HDL cholesterol levels in the bottom tenth percentile and a homozygote has no measurable HDL cholesterol (11). A heterozygote for an internal 15-residue deletion in apoA-I has an HDL cholesterol of only 7 mg/dl and an apoA-I level of 14 mg/dl (12). Finally, heterozygotes for apoA-I_{Milano}, which contains a cysteine for arginine substitution at position 173, have HDL levels approximately one-third of normal (6). When studied in vivo, both apoA-I_{Milano} and normal apoA-I were found to have increased fractional catabolic rates in apoA-I_{Milano} subjects compared with normal apoA-I in normal subjects (29).

Heterozygotes for apoA-I_{Iowa} also have low plasma levels of HDL and apoA-I. This study establishes that the hypoalphalipoproteinemia in heterozygous apoA-I_{Iowa} subjects is due to accelerated catabolism of both normal and mutant apoA-I. These results indicate that the presence of apoA-I_{Iowa} affects the catabolism of normal apoA-I. Although the total pool size of apoA-I in the heterozygotes is somewhat smaller and may affect the rate of normal

TABLE 3. Kinetic parameters of apoA-I metabolism in apoA-I_{Iowa} and control subjects

Subject		ApoA-I_{normal} Conc	ApoA-I_{normal} FCR	ApoA-I_{normal} PR	ApoA-I_{Iowa} Conc	ApoA-I_{Iowa} FCR	ApoA-I_{Iowa} PR	Total[a] PR
		mg/dl	*d^{-1}*	*mg/kg-d*	*mg/dl*	*d^{-1}*	*mg/kg-d*	*mg/kg-d*
ApoA-I_{Iowa}	1	49	0.278	5.5	20	0.589	4.7	10.2
ApoA-I_{Iowa}	2	52	0.283	5.9	21	0.565	4.8	10.7
Control	1	119	0.211	10.0		0.606		10.0
Control	2	133	0.204	10.9		0.474		10.9
Historical controls (n = 9)								
	Mean	124	0.203	10.1				10.1
	± SD	15	0.016	1.1				1.1

[a]Total PR = apoA-I_{normal} PR + apoA-I_{Iowa} PR.

apoA-I catabolism, it is more likely that the apoA-I_{Iowa} has a direct effect on normal apoA-I. The shift in distribution from HDL_2 to HDL_3 of both forms of radiolabeled apoA-I in the apoA-I_{Iowa} heterozygotes suggests that apoA-I_{Iowa} may result in smaller HDL particles, which in turn may cause more rapid catabolism of normal apoA-I (30). Alternatively, the presence of apoA-I_{Iowa} on HDL particles may alter their interaction with cells and directly result in faster catabolism of associated normal apoA-I. ApoA-II levels are also low in the apoA-I_{Iowa} subjects, suggesting that apoA-II may be more rapidly catabolized as well.

The FCR of apoA-I_{Iowa} was more than twice as fast as that of normal apoA-I in both the heterozygous subjects and the controls. Though the mutant apoA-I_{Iowa} protein was rapidly removed from plasma, its associated radioactivity did not appear cumulatively in the urine at the rate to be expected if the protein were following normal catabolic pathways. Instead, the apoA-I_{Iowa} protein appeared to accumulate in extravascular tissues. This predilection to be rapidly removed from plasma and sequestered in extravascular tissues was shown to be an intrinsic property of this mutant apolipoprotein, as its metabolic behavior was similar even when injected into normal subjects.

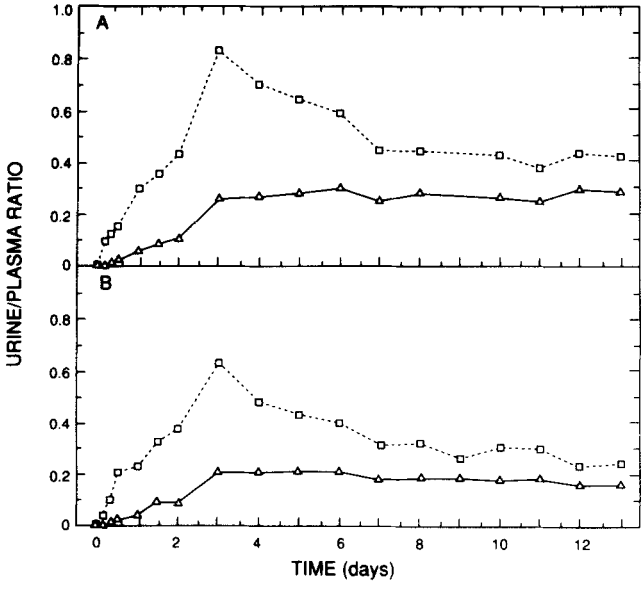

Fig. 4. Urine/plasma radioactivity ratios for apoA-I_{Iowa} (squares and dotted lines) and normal apoA-I (triangles and solid lines). A) ApoA-I_{Iowa} subject #1; B) normal subject #1.

The mechanism by which the 83-residue amino-terminal portion of apoA-I_{Iowa} is generated and only this fragment sequestered and incorporated into amyloid fibrils remains uncertain. Residue 83 of apoA-I is an arginine, and many plasma serine proteases are specific for the peptide bond at the carboxylic side of arginine residues. It has previously been demonstrated that proteolysis of certain mutant proteins can generate amyloidogenic β-pleated sheet peptides (31). It remains to be determined whether the gly to arg mutation at residue 26 makes the apoA-I_{Iowa} protein more susceptible to specific proteolytic cleavage, and if so, whether this occurs within the plasma or at the site of tissue deposition. In either case, the fragment consisting of the carboxy-terminal two thirds of apoA-I generated by the proteolytic cleavage is not sequestered in extravascular tissues. This fragment is probably rapidly catabolized, likely accounting for the majority of the urinary excretion of apoA-I_{Iowa}-associated radioactivity and the marked heterogeneity of metabolism seen on the U/P ratio curves.

The difference in turnover rates between apoA-I_{Iowa} and normal apoA-I within the same subject was more pronounced in HDL_3 than in HDL_2, and most marked in the $d > 1.21$ g/ml fraction. This observation has several potential explanations. First, apoA-I_{Iowa} may result in the formation of small dense HDL particles that cannot become normally cholesterol-enriched and are therefore rapidly catabolized. Second, it may self-associate within the plasma, resulting in lipid-poor aggregates that are rapidly cleared. Finally, the carboxy-terminal apoA-I fragment generated by the proteolytic clip may not associate normally with HDL particles and therefore may be

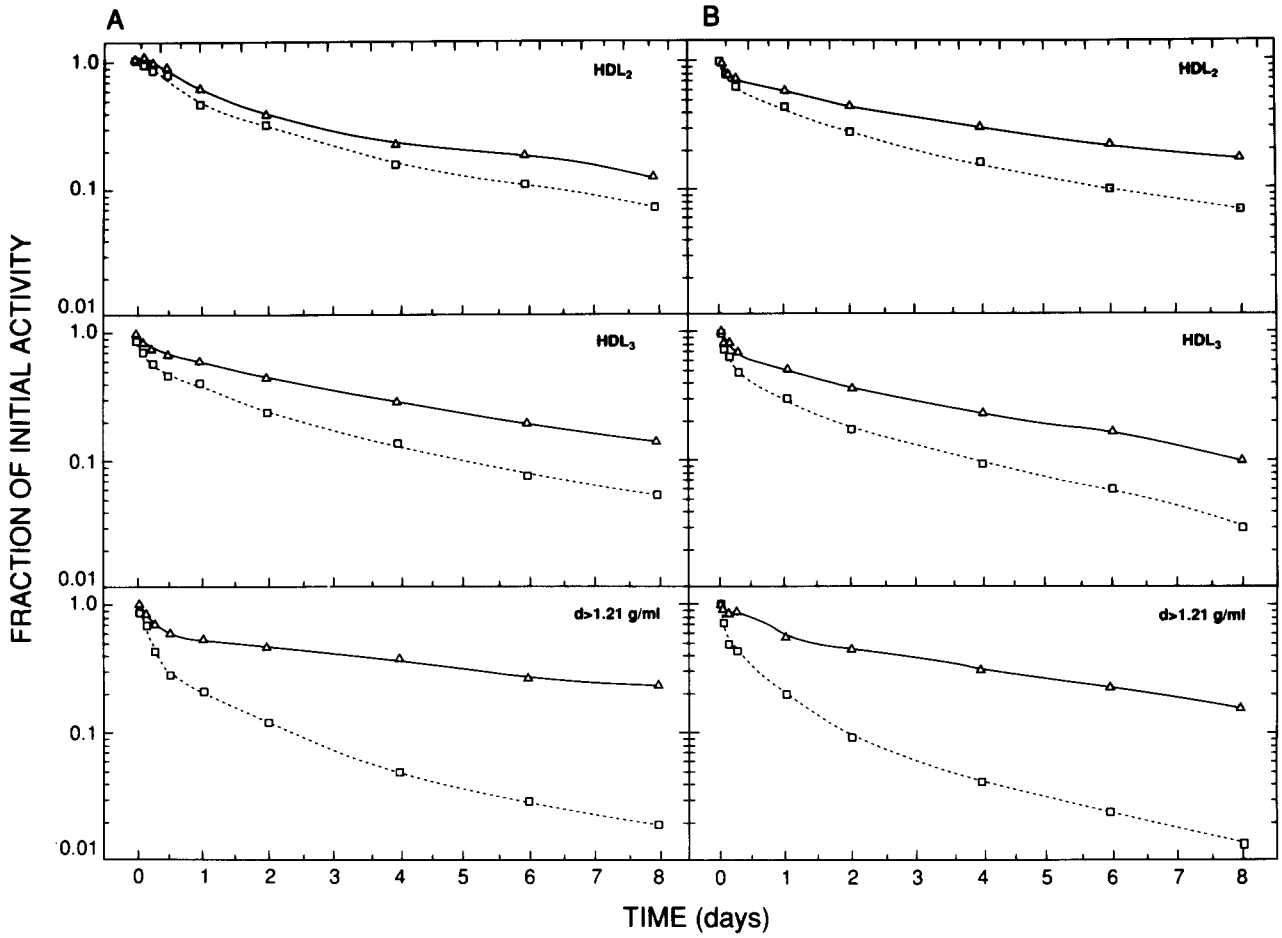

Fig. 5. Turnover of apoA-I_{Iowa} (squares and dotted lines) and normal apoA-I (triangles and solid lines) in lipoprotein density fractions. A) ApoA-I_{Iowa} subject #1; B) normal subject #1.

more likely to be found in the d > 1.21 g/ml fraction, from which it may be rapidly catabolized. Further characterization of the lipoprotein particles in these subjects will be required to address these possibilities.

The markedly abnormal metabolism of the apoA-I_{Iowa} protein suggests that the single amino acid substitution has a significant effect on the structure of the mutant protein. The 64-residue amino-terminus of apoA-I is highly conserved across several species (32–38). The glycine at residue 26 of the mature apolipoprotein is completely conserved in all seven species in which apoA-I has been sequenced. A model of protein secondary structure (39) predicts apoA-I to have an amino-terminus that is largely in β-pleated sheet conformation, as has been previously demonstrated for other amyloidogenic proteins (40). Normal apoA-I is predicted to have a β-turn at residues 24 to 27, whereas the gly_{26} to arg substitution in apoA-I_{Iowa} is predicted to eliminate this turn; this could significantly alter the physico-chemical properties and metabolic behavior of this mutant apolipoprotein. Recently described was a patient with apoA-I deficiency who is homozygous for a codon 84 nonsense mutation in apoA-I (28), predicting synthesis of a truncated 83-residue peptide. It is not known whether this peptide is secreted; however the patient has no clinical evidence of amyloidosis, suggesting that an 83-residue amino-terminal peptide of normal apoA-I may not itself be amyloidogenic.

In conclusion, the single amino acid substitution of gly_{26} to arg in apoA-I_{Iowa} results in strikingly aberrant metabolism in which the mutant apolipoprotein is rapidly removed from plasma, with a portion abnormally sequestered in tissues while the remainder is rapidly catabolized and degraded. In addition, heterozygosity for apoA-I_{Iowa} is associated with accelerated catabolism of normal apoA-I. These results explain the low levels of HDL and apoA-I and further the understanding of the systemic amyloidosis in heterozygotes for the apoA-I_{Iowa} mutation.

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