Human apolipoprotein A-I isoprotein metabolism: proapoA-I conversion to mature apoA-I [1]

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Abstract ProapoA-I (apoA-I_{+2} isoform) is the major apoA-I isoprotein secreted by the liver and intestine; however, it is a minor isoprotein in plasma and lymph where the major A-I apolipoprotein is mature apoA-I (apoA-I_0, apoA-I_{-1}, and apoA-I_{-2} isoforms). In the present report we provide evidence that apoA-I is rapidly and quantitatively converted to mature apoA-I, and the mature apoA-I isoforms are catabolized at equal rates. In these studies, human proapoA-I was isolated from thoracic duct chylomicrons collected during active fat absorption and mature apoA-I was isolated from plasma high density lipoproteins. The isolated lipoproteins were delipidated, fractionated by gel permeation chromatography, and the individual apoA-I isoforms were separated by preparative isoelectrofocusing. The metabolism of apoA-I isoproteins was studied in normal volunteers (N = 6) in a metabolic ward. In the first study proapoA-I and mature apoA-I (apoA-I_0 isoform) were injected simultaneously into two normal subjects and the conversion of proapoA-I to mature apoA-I and the decay of radioactivity were followed in plasma and HDL over a 14-day period. ProapoA-I was rapidly and completely converted to mature apoA-I with a fractional rate of conversion of 4.0 pools/day. The average residence times of proapoA-I and mature apoA-I were 0.23 and 6.5 days, respectively. The mature apoA-I derived from proapoA-I had a residence time which was the same as the injected mature apoA-I. The production rates of proapoA-I and mature apoA-I were virtually identical in the subjects studied, indicating that essentially all of mature apoA-I was derived from proapoA-I. In the second study, the catabolism of the mature apoA-I isoforms, apoA-I_0, apoA-I_{-1}, and apoA-I_{-2}, was analyzed by simultaneous injection of apoA-I_0 and apoA-I_{-1} in two subjects, and apoA-I_0 and apoA-I_{-2} in two subjects. The radioactivity decay in plasma of the three mature apoA-I isoforms was identical, indicating that there were no differences in the catabolism of the mature isoforms. Analysis of the individual radiolabeled mature apoA-I isoforms within HDL by preparative isoelectrofocusing revealed that apoA-I_0 and apoA-I_{-1} isoforms were slowly converted to the apoA-I_{-1} and apoA-I_{-2} isoforms, respectively, during the 14-day study. The combined results from these studies indicate that proapoA-I was rapidly and completely converted to mature apoA-I, mature apoA-I was derived essentially completely from proapoA-I, and the isoforms of mature apoA-I have similar catabolic rates. The extracellular post-translational processing of apoA-I provides a new site for modulation of apoA-I function and metabolism. —**Bojanovski, D., R. E. Gregg, G. Ghiselli, E. J. Schaefer, J. A. Light, and H. B. Brewer, Jr.** Human apolipoprotein A-I isoprotein metabolism: proapoA-I conversion to mature apoA-I. *J. Lipid Res.* 1985. **26:** 185–193.

Supplementary key words isoelectric focusing • high density lipoproteins

Human apolipoprotein A-I, a major protein constituent of HDL, contains 243 amino acids and is secreted primarily by the liver and small intestine (1–6). Initial studies on the in vitro biosynthesis of apoA-I by fetal or adult intestine, fetal liver, and a hepatoma cell line indicated that newly synthesized apoA-I was a more basic isoprotein than plasma apoA-I (7–9). These data suggested a conversion of newly synthesized apoA-I to circulating plasma apoA-I; however, the nature of this conversion which resulted in a net change of two positive charges was unknown.

Recent advances in our understanding of the biosynthesis and processing of human apoA-I have permitted a more definitive analysis of the metabolic conversion and catabolism of plasma apoA-I. Human apoA-I is synthesized as a 267 amino acid precursor, preproapoA-I (10–14) which undergoes co-translational cleavage to proapoA-I. ProapoA-I contains a hexapeptide, arg-his-phe-trp-gln-gln, attached to the amino-terminus of apoA-I. Recent studies from our laboratory have established that proapoA-I can be isolated from human plasma or lymph and is located at the apoA-I_{+2} position on electrophoretograms of plasma separated by two-dimensional gel electrophore-

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Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoprotein; $NaDodSO_4$, sodium dodecyl sulfate.

[1]Presented in part at the Annual Scientific Sessions of the American Society for Clinical Investigation, May 1, 1983.

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sis (15, 16). The amino acid sequence of the propeptide of proapoA-I (15) isolated from thoracic duct lymph was identical to the sequence determined by nucleic acid sequence analysis of cloned apoA-I (10–14).

The presence of human proapoA-I in plasma and lymph suggested that proapoA-I might be converted to mature apoA-I in plasma in vivo. To date, no definitive evidence on the metabolic conversion of the apoA-I isoproteins has been available in humans. The present study was undertaken to directly investigate the plasma metabolism of human proapoA-I isolated from thoracic duct lymph.

MATERIALS AND METHODS

Apolipoprotein isolation and characterization

ApoA-I isoforms in plasma, lymph, or isolated lipoprotein density fractions have been routinely assessed by two-dimensional gel electrophoresis as previously reported (7–9, 15–17). The nomenclature now utilized for apoA-I isoforms as reviewed previously (17) conforms to the nomenclature standardized for proteins separated by two-dimensional gel electrophoresis (18). In this system established by Anderson and Anderson (18, 19) the two-dimensional electrophoretograms are presented with the acidic and basic regions of the gel to the left and right, respectively, and the major unsialylated protein isoform is designated with a zero subscript. Other isoforms of the protein are codified by unit charge (− or +) from the major unsialylated-sialylated isoform. Thus, the major mature apoA-I isoform is designated apoA-I_0, and the minor mature isoforms, apoA-I_{-1} and apoA-I_{-2}. ProapoA-I, which contains two additional positive charges when compared to mature apoA-I, is located at the apoA-I_{+2} position on the electrophoretogram. The apoA-I isoform apoA-I_0 has been previously designated apoA-I_3 (15, 16), apoA-I_4 (7), or apoA-I_1 (20).

ProapoA-I was isolated from lymph chylomicrons by centrifugation of thoracic duct lymph in a Beckman SW27 swinging bucket rotor at 25,000 rpm at 5°C for 120 min. Chylomicrons were resuspended in a NaCl solution at a final density of 1.006 g/ml and recentrifuged three times under identical conditions as outlined above. Following the last recentrifugation the chylomicrons were dispersed in 0.08 M NH_4HCO_3 (pH 8.2), lyophilized, and delipidated by three extractions with chloroform–methanol 3:1. The delipidated apolipoproteins (19 mg) were dissolved in 0.15 M NaCl, 0.05 M Tris-HCl, 8 M urea (pH 8.2) and fractionated on a Sephacryl S-200 column (2.4 × 170 cm, Pharmacia) equilibrated in the same buffer. The flow rate was 30 ml/hr and 3-ml fractions were collected. The apolipoprotein composition of the eluted fractions was determined by analytical $NaDodSO_4$ polyacrylamide gel electrophoresis as previously described (6). The fractions containing apoA-I were pooled and extensively dialyzed against 0.01 M NH_4HCO_3 (pH 8.2), lyophilized, and fractionated by preparative isoelectrofocusing in a vertical slab gel apparatus (Bio-Rad, Richmond, CA) utilizing a 3-mm thick gel with a pH gradient from 5.0 to 5.5 (Serva, Heidelberg, FRG) (21). After electrophoresis the area of the gel corresponding to proapoA-I (apoA-I_{+2} isoform) was identified by staining a lateral portion of the gel, and the purified apolipoprotein was recovered from the unstained portion by cutting the gel, followed by electrophoretic elution into a dialysis bag in 0.01 M NH_4HCO_3 at 4°C for 12 hr. The purified isoprotein was separated from low molecular weight contaminants by gel filtration on a Sephadex G-100 column (1.2 × 50 cm, Pharmacia) equilibrated in 0.01 M NH_4HCO_3 (pH 8.2).

Mature apoA-I was isolated from HDL obtained from fasting normal subjects following sequential ultracentrifugation in a Beckman 60 Ti rotor (Beckman Inst., Palo Alto, CA) between densities 1.063 g/ml and 1.210 g/ml (22). The isolated HDL was recentrifuged at 1.210 g/ml, dialyzed extensively against 0.01 M NH_4HCO_3 (pH 8.2), lyophilized, and delipidated with chloroform–methanol 3:1 as described above. The delipidated apolipoproteins (100 mg) were dissolved and fractionated by gel permeation chromatography over a Sephacryl S-200 column (4.0 × 190 cm, Pharmacia) in a buffer containing 8 M urea and 0.05 M Tris-HCl (pH 8.2). The fractions containing apoA-I were identified by $NaDodSO_4$ gel electrophoresis, pooled, and dialyzed against 0.01 M NH_4HCO_3 (pH 8.2). Each of the isoforms of mature apoA-I (apoA-I_0, apoA-I_{-1}, and apoA-I_{-2}) were isolated by preparative isoelectrofocusing as outlined above.

Purified proapoA-I and the purified isoforms of mature apoA-I were analyzed for purity by two-dimensional gel electrophoresis, $NaDodSO_4$ gel electrophoresis, and urea polyacrylamide gel electrophoresis (6, 16, 17).

ApoA-I in plasma was quantitated by radioimmunodiffusion as previously reported (23). ProapoA-I was quantified from the concentration of plasma apoA-I multiplied by the percent of proapoA-I of total apoA-I determined on two-dimensional gel electrophoretograms of plasma (17). In normal plasma proapoA-I represents 4.0 ± 0.2% (n = 4) of total apoA-I.

Apolipoprotein iodination

ProapoA-I and apoA-I were iodinated by a modification of the McFarlane method (23) in 6 M guanidine-HCl (pH 8.2). More than 97% of the apolipoprotein-associated radioactivity was located in either the proapoA-I or mature apoA-I bands when analyzed by $NaDodSO_4$ electrophoresis.

To determine the distribution of radioactivity among

the different apoA-I isoproteins, radiolabeled proapoA-I and the mature apoA-I isoforms were added in trace quantities to delipidated HDL dissolved in 8 M urea just prior to fractionation by isoelectrofocusing. The portion of the gel containing the appropriate band was identified, cut, and the radioactivity was quantitated in a Packard model 5260 Autogamma Spectrometer (Packard Instrument Co., Inc., Downers Grove, IL).

Prior to injection for metabolic studies, radiolabeled apolipoproteins were incubated in normal plasma for 30 min at 25°C after which the plasma was adjusted to 1.21 g/ml with solid KBr and the 1.21-g/ml supernate was isolated by ultracentrifugation as outlined above. The isolated lipoprotein fractions were dialyzed against 0.1 M Tris-HCl, 0.15 M NaCl, 0.01% EDTA, pH 7.4, passed through a 0.22-μm Millipore filter (Millipore, Bedford, MA), and tested for sterility and pyrogenicity (23). All preparations were sterile, pyrogen-free, and no free iodine was detected by radiochromatography (85% methanol on Whatman Number 1 filter paper, Whatman Co., Clifton, NJ) utilizing a Packard Model 7201 radiochromatogram scanner (Packard Instrument Co., Inc.).

Metabolic studies

Normal volunteers (n = 6) were admitted to the metabolic ward 1 week prior to the studies and placed on a diet containing 42% of calories as fat (polyunsaturated: saturated fat ratio 0.2), 42% carbohydrate, and 16% protein. All subjects had normal thyroid, liver, and kidney function. Each subject received supersaturated potassium iodide solution (0.9 g/day) in divided doses 1 day prior to, and during the course of the study. The metabolic studies were performed under an approved NHLBI peer-reviewed protocol for the injection of radiolabeled apolipoproteins and lipoproteins in humans. Informed consent was obtained from all subjects.

Total plasma cholesterol, plasma triglycerides, and HDL cholesterol levels, as well as plasma and HDL apoA-I concentrations, were determined on admission and during the course of the study as previously described (23). Cholesterol and triglyceride concentrations were determined on a Gilford 3500 automated system (Gilford, Oberlin, OH), and the plasma lipoprotein fractionations were performed by the procedures standardized by the Lipid Research Clinics (24).

The following studies were performed:

Study A. ^{125}I-proapoA-I (apoA-I_{+2}) (50 μCi) and mature ^{131}I-apoA-I (apoA-I_0) (25 μCi) were simultaneously injected into two normal volunteers. Blood samples were collected in 0.1% EDTA at 10 min, 3, 6, 12, 18, and 24 hr, and then daily for 14 days. Radioactivity was determined in whole plasma and HDL. In addition, HDL isolated from plasma collected at 10 min, 3 hr, 6 hr, 12 hr, 18 hr, and at days 1, 2, 3, 4, 5, 7, 9, 11, and 14 following injection was used for the determination of the distribution of radioactivity among the apoA-I isoforms. HDL isolated at the indicated time points was dialyzed against 0.01 M NH_4HCO_3 (pH 8.2), lyophilized, delipidated, and fractionated by isoelectrofocusing on a vertical slab gel apparatus (Bio-Rad, 3-mm thick, pH 4 to 6, Serva Ampholines, Heidelberg, FRG) as described above. From 0.2 to 1 mg of apoHDL was applied to each gel. After staining, the portions of the gel containing each of the apoA-I isoforms were cut with a razor blade, and the radioactivity was quantitated. The radioactivities within isoform bands apoA-I_{+1} and apoA-I_{+2} were pooled for proapoA-I, and the three mature apoA-I isoforms, apoA-I_0, apoA-I_{-1}, and apoA-I_{-2}, were pooled for mature apoA-I (21). Radioactivities for the isoproteins were calculated by relating the percent radioactivity within isoform bands to total apoA-I radioactivity.

Study B. Four normal volunteers were studied in order to analyze the metabolism of the mature apoA-I forms. Two subjects were injected with ^{125}I-apoA-I_0 (50 μCi) and ^{131}I-apoA-I_{-1} (25 μCi) and two subjects received ^{125}I-apoA-I_0 (50 μCi) and ^{131}I-apoA-I_{-2} (25 μCi). Blood samples were collected in 0.1% EDTA and the radioactivity was quantitated in plasma, isolated lipoprotein density fractions, and the apoA-I isoforms of HDL separated by preparative isoelectrofocusing as outlined for study A.

Calculation of kinetic parameters

The plasma residence times for the injected apolipoproteins were computed utilizing the SAAM 27 program (25, 26) on a VAX 11/780 computer (Digital Equipment Co., Maynard, MA).

RESULTS

Apolipoprotein isolation

We have previously reported that thoracic duct lymph obtained during active fat absorption contains an increased concentration of proapoA-I (16). ProapoA-I has been isolated from lymph lipoproteins ($d < 1.006$ g/ml) and mature apoA-I isoforms (apoA-I_0, apoA-I_{-1}, and apoA-I_{-2}) from HDL by gel permeation chromatography followed by preparative isoelectrofocusing. The purity of the isolated proapoA-I and the mature apoA-I isoforms is illustrated in the two-dimensional electrophoretograms in **Fig. 1.** We have previously established, by amino-terminal sequence analysis of apoA-I_{+2} from thoracic duct lymph, that this isoform is proapoA-I (15).

Characterization of radioiodinated apolipoproteins

The efficiency of iodination of the different apoA-I isoforms varied from 35 to 50% and there was less than 1 mole of iodine covalently bound per 2 moles of apoA-I.

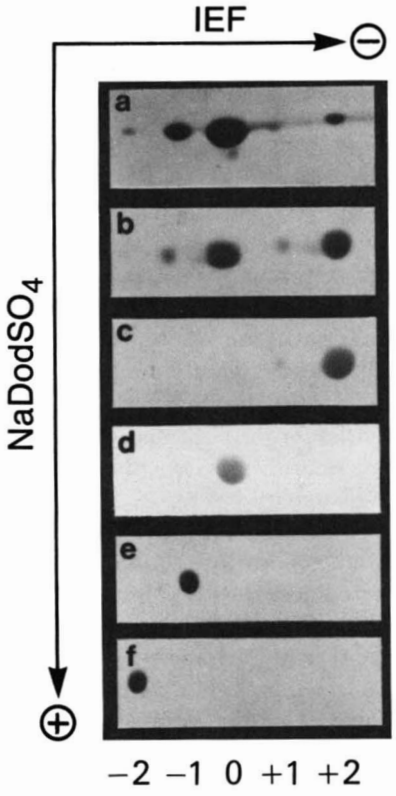

Fig. 1 Two-dimensional gel electrophoretograms of apoA-I isoforms in plasma and lymph as well as the isolated apoA-I isoforms. Only the portion of the gel showing the apoA-I isoforms is presented. Individual panels include: a) plasma; b) lymph chylomicrons; c) apoA-I_{+2} (proapoA-I); d) apoA-I_0 isoform; e) apoA-I_{-1} isoform; f) apoA-I_{-2} isoform.

Radioiodinated apolipoproteins retained their immunological properties when analyzed by immunoprecipitation. On analysis by monodimensional isoelectrofocusing, 83.3% of ^{131}I-proapoA-I was present in the apoA-I_{+2} isoform. ^{125}I and ^{131}I radiolabeled apoA-I mature isoforms contained 86.7 ± 1.5% (mean ± SEM, n = 4) of the radioactivity in the respective radiolabeled isoforms. Utilizing this procedure, there was no incompetent apoA-I monomer when analyzed by high pressure liquid chromatography (27).

Metabolic studies

The concentrations of plasma cholesterol, plasma triglycerides, HDL cholesterol and apoA-I of the study subjects are given in **Table 1.** In order to evaluate the conversion of proapoA-I to mature apoA-I, ^{125}I-proapoA-I (apoA-I_{+2}) and mature ^{131}I-apoA-I (apoA-I_0) were simultaneously injected into two normal volunteers and the radioactivity decay was determined in plasma and HDL (Study A). The distribution of ^{125}I-proapoA-I and mature ^{131}I-apoA-I radioactivity in the individual isoforms was determined in HDL following separation by isoelectrofocusing. ^{125}I-proapoA-I rapidly disappeared from plasma, and there was a corresponding increase in ^{125}I radioactivity in mature apoA-I (**Fig. 2**). The specific activity curves of ^{125}I-proapoA-I and mature apoA-I intersected at the peak of the ^{125}I-labeled mature apoA-I curve (Fig. 2, inset) and the ^{125}I-labeled mature apoA-I derived from ^{125}I-proapoA-I decayed at a rate virtually identical to mature ^{131}I-apoA-I injected simultaneously with the ^{125}I-proapoA-I (Fig. 2). These results are interpreted as indicating that proapoA-I is the major, if not only, pool from which mature apoA-I is derived since the specific activity curve of proapoA-I crosses the mature apoA-I specific activity curve at its peak, thus indicating a simple precursor-product relationship. In addition, proapoA-I was quantitatively converted to mature apoA-I. Incomplete conversion of proapoA-I to mature apoA-I would have been reflected as a reduced but parallel curve of the derived mature apoA-I compared to the injected mature apoA-I when plotted as fraction of injected dose. Since these two curves were virtually superimposable, this indicates complete conversion of proapoA-I to mature apoA-I (Fig. 2).

The kinetic parameters of proapoA-I and mature apoA-I metabolism analyzed in Study A are shown in **Table 2.** The plasma residence times of proapoA-I were rapid and averaged 0.23 days, as compared to the residence time of 6.5 days for mature apoA-I. The residence time of the apoA-I_0 isoform of mature apoA-I analyzed in normal subjects in this study is similar to the residence times determined on mature apoA-I in our previous studies (23). The production rates of proapoA-I and mature apoA-I determined from ^{125}I-proapoA-I and ^{131}I-apoA-I kinetics were 8.8 and 7.5 mg/kg-day in subject 1 and 11.6 and 10.2 mg/kg-day in subject 2, respectively (Table 2). The combined results from this study establish that proapoA-I is rapidly and essentially completely converted to mature apoA-I. In addition, the similarity in the production rates of proapoA-I and mature apoA-I in each subject indicates that virtually all mature apoA-I is derived from proapoA-I.

To further define the metabolism of mature apoA-I, a second study (Study B) was performed on the purified mature apoA-I isoforms. The isolated mature isoforms were radiolabeled as follows: ^{125}I-apoA-I_0, ^{131}I-apoA-I_{-1} and ^{131}I-apoA-I_{-2}. Two subjects were injected simultaneously with ^{125}I-apoA-I_0 and ^{131}I-apoA-I_{-1}, and two subjects were injected with ^{125}I-apoA-I_0 and ^{131}I-apoA-I_{-2}. The decay of radioactivity was followed in plasma and HDL for 14 days.

The plasma decay curves of ^{125}I-apoA-I_0 and ^{131}I-apoA-I_{-1} in the two subjects were identical (**Fig. 3**). The plasma decay curves in the two subjects who received ^{125}I-apoA-I_0 and ^{131}I-apoA-I_{-2} were also identical (**Fig. 4**). These results establish that all three of the major mature apoA-I isoforms were catabolized at similar rates.

In order to determine whether there was a conversion between the individual mature apoA-I isoforms, the dis-

TABLE 1. Plasma lipids, apolipoprotein A-I, and HDL cholesterol concentrations

Subjects	Age	Sex	Study	Injected Tracer	Plasma Cholesterol[a]	Plasma Triglycerides[a]	HDL Cholesterol[a]	Plasma ApoA-I[b]
					mg/dl			
1	20	M	A	^{125}I-ProapoA-I (apoA-I_{+2} isoform) ^{131}I-Mature apoA-I (apoA-I_0 isoform)	232	69	76	149 ± 2
2	23	F	A	^{125}I-ProapoA-I (apoA-I_{+2} isoform) ^{131}I-Mature apoA-I (apoA-I_0 isoform)	182	59	86	139 ± 3
3	20	M	B	^{125}I-Mature apoA-I (apoA-I_0 isoform) ^{131}I-Mature apoA-I (apoA-I_{-1} isoform)	126	75	55	128 ± 1
4	22	F	B	^{125}I-Mature apoA-I (apoA-I_0 isoform) ^{131}I-Mature apoA-I (apoA-I_{-1} isoform)	185	82	49	125 ± 4
5	19	M	B	^{125}I-Mature apoA-I (apoA-I_0 isoform) ^{131}I-Mature apoA-I (apoA-I_{-2} isoform)	208	162	75	142 ± 2
6	20	F	B	^{125}I-Mature apoA-I (apoA-I_0 isoform) ^{131}I-Mature apoA-I (apoA-I_{-2} isoform)	207	74	55	120 ± 3

[a]Concentrations for the different subjects at admission.
[b]Mean apoA-I ± SEM value for each time point during the metabolic studies; n = 12 for Study A; n = 11 for Study B.

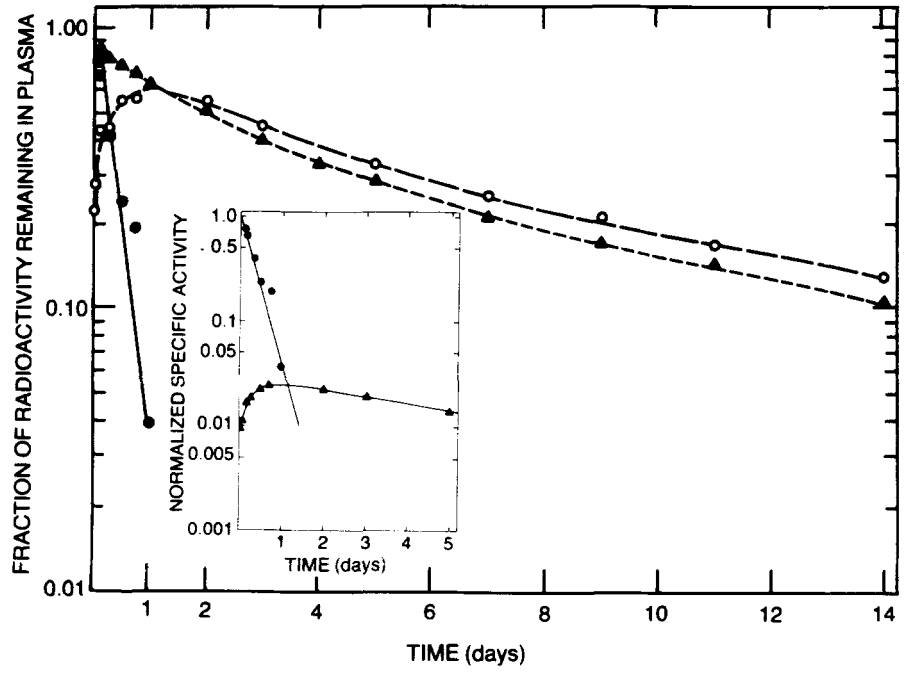

Fig. 2 Plasma radioactivity decay, expressed as fraction of injected dose, of ^{125}I-proapoA-I and ^{131}I-apoA-I_0 injected simultaneously in a normal volunteer. The apoA-I isoforms were separated in HDL by preparative isoelectrofocusing. ^{125}I-proapoA-I (●—●) was injected and the mature ^{125}I-apoA-I (○—○) derived from ^{125}I-proapoA-I was compared with the kinetics of mature ^{131}I-apoA-I_0 injected simultaneously (▲—▲). The insert illustrates the specific activity curves of proapoA-I (●—●) and mature apoA-I (▲—▲) derived from proapoA-I during the 14-day study.

TABLE 2. Kinetic parameters of apoA-I isoprotein metabolism

Subject	ProapoA-I	Residence Time	Production Rate	Mature ApoA-I	Residence Time	Production Rate
	mg/dl	*days*	*mg/kg-day*	*mg/dl*	*days*	*mg/kg-day*
1	6.0	0.27	8.8	143	7.7	7.5
2	5.5	0.19	11.6	133	5.2	10.2

tribution of radioactivity and metabolic conversions of ^{125}I-apoA-I$_0$ were analyzed in HDL by preparative isoelectrofocusing and quantitation of radioactivity within the major mature apoA-I isoforms. Eighty-seven percent of the ^{125}I-apoA-I$_0$ tracer was in the apoA-I$_0$ isoform band (**Table 3**). Following injection the radioactivity within the apoA-I$_0$ isoform slowly decreased to 72% and increased in apoA-I$_{-1}$ to 28% by day 14 (Table 3) with a fractional conversion rate of 0.013 pools per day. A very similar slow increase in radioactivity in apoA-I$_{-2}$ was observed following the injection of ^{131}I-apoA-I$_{-1}$ (data not shown). These data indicate that there is a slow conversion of apoA-I$_0$ to apoA-I$_{-1}$ and apoA-I$_{-1}$ to apoA-I$_{-2}$.

DISCUSSION

The significance of the polymorphism of plasma apoA-I in man has recently been the subject of active research (7–17). ApoA-I polymorphism consists of a series of isoproteins of which apoA-I$_0$ and apoA-I$_{-1}$ are the major isoforms. The pattern of apoA-I isoproteins is quite constant within the general population, suggesting both little allelic variation of apoA-I and a specific metabolic cascade of apoA-I metabolism. The liver and the small intestine are the major sites of synthesis of apoA-I (1–5). The most basic human apoA-I isoform (apoA-I$_{+2}$) which has been shown to be proapoA-I (15) is the major isoprotein secreted by fetal or adult intestine, fetal liver, and a human hepatoma cell line (7–9). These observations strongly suggested that newly synthesized proapoA-I is rapidly converted by a post-translational cleavage to the mature apoA-I isoforms and a proapoA-I to mature apoA-I converting activity has been demonstrated in vitro (21, 28). However, direct evidence for the metabolic conversion of proapoA-I to mature apoA-I in plasma in man has not been established. Specifically, no definitive data have been presented to establish that proapoA-I was secreted from the liver or intestines in vivo in man. Secondly, no in vivo data have been reported to establish that proapoA-I was converted to other apoA-I isoproteins in human plasma. Recently our laboratory has isolated apoA-I$_{+2}$ from human lymph and has determined by amino acid sequence analysis that proapoA-I was present in human plasma and lymph (15). In addition, proapoA-I was shown to be a major apoA-I constituent in the d < 1.006 g/ml lipoprotein fraction of human lymph. The concentration of proapoA-I significantly increased following fat absorp-

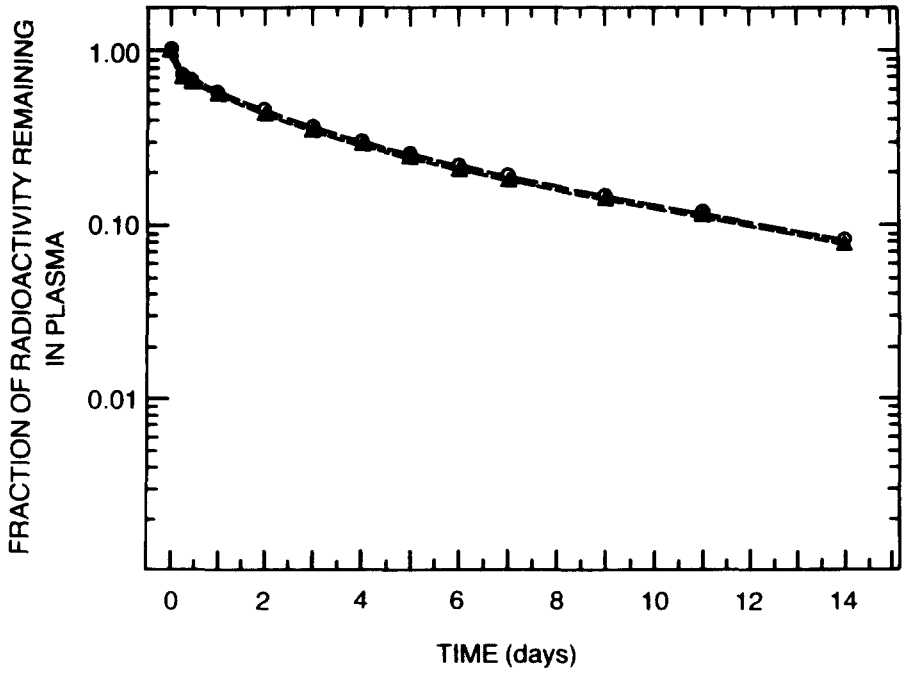

Fig. 3 Decay of plasma radioactivity of ^{125}I-apoA-I$_0$ (▲—▲) and ^{131}I-apoA-I$_{-1}$ (○—○) injected simultaneously into a normal volunteer. After the injection of radiolabeled apolipoproteins, plasma samples were obtained at 10 min (0 study time point), 3, 6, 12, and 24 hr, and then daily until 14 days.

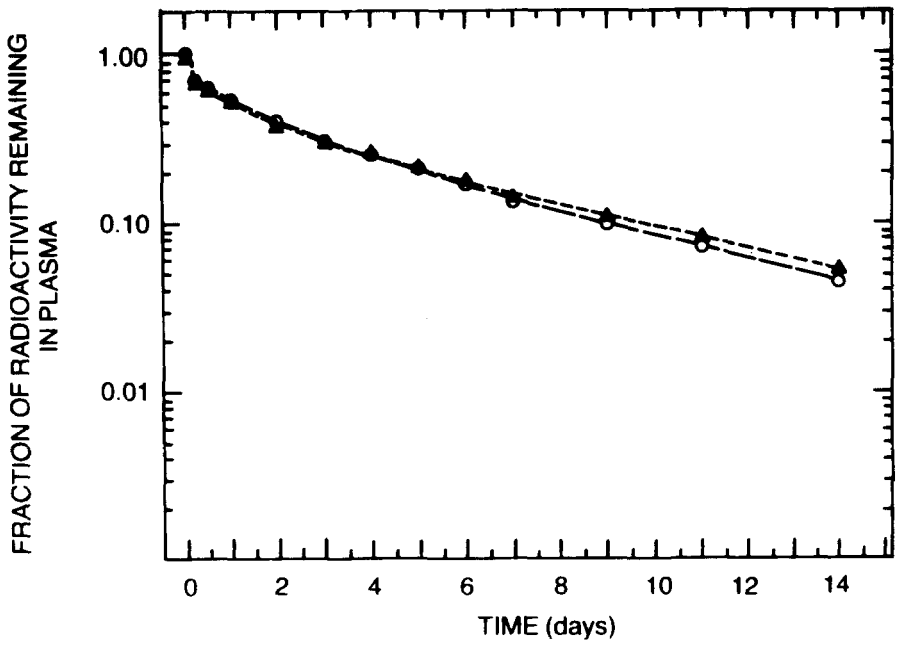

Fig. 4 Decay of plasma radioactivity of ^{125}I-apoA-I$_0$ (▲—▲) and ^{131}I-apoA-I$_{-2}$ (○—○) injected simultaneously into a normal volunteer. After the injection of radiolabeled apolipoproteins, plasma samples were obtained at 10 min (0 study time point), 3, 6, 12, and 24 hr, and then daily until 14 days.

tion, supporting the concept that the biosynthesis of proapoA-I is modulated by the state of intestinal absorption and under physiological control (16).

The studies reported here address the question of the conversion of proapoA-I to mature apoA-I in vivo. The kinetic analysis of radiolabeled proapoA-I and mature apoA-I injected into normal subjects definitively established that proapoA-I was rapidly and virtually completely converted to mature apoA-I in plasma in vivo. The catabolic rate of mature apoA-I generated from proapoA-I was identical to that of radiolabeled mature apoA-I injected directly into plasma. The synthesis rates of proapoA-I and mature apoA-I were nearly identical indicating that the mature apoA-I was derived essentially completely from proapoA-I.

Whether the conversion of proapoA-I to apoA-I occurs predominately in plasma and lymph, on a particular type of lipoprotein particle, or at specific tissue sites has not been established; however, cleavage of proapoA-I to apoA-I occurs rapidly. In addition, newly synthesized apoA-I associated with chylomicrons is rapidly transferred to HDL in man (29), but it is as yet unknown whether it is transferred as proapoA-I or mature apoA-I. The physiological importance of proapoA-I in apoA-I metabolism has yet to be established, and it will be of importance to determine whether the cleavage of proapoA-I to mature apoA-I is associated with the initiation or the termination of a specific function(s).

Analyses of the kinetics of the individually radiolabeled mature apoA-I isoforms (apoA-I$_0$, apoA-I$_{-1}$, and apoA-I$_{-2}$) were identical establishing that there was no significant difference in the catabolism of the major apoA-I isoforms. There was, however, a slow conversion of apoA-I$_0$ to apoA-I$_{-1}$ and of apoA-I$_{-1}$ to apoA-I$_{-2}$ consistent with deamidation of the isoforms during circulation in plasma which did not effect the catabolic rate.

A schematic overview of the biosynthesis, conversion, and catabolism of proapoA-I and mature apoA-I is illustrated in **Fig. 5.** ApoA-I is secreted as proapoA-I and converted rapidly to mature apoA-I, which is catabolized

TABLE 3. Percent distribution of radioactivity among mature apoA-I isoforms following injection of ^{125}I-apoA-I$_0$

Time	Mature ApoA-I Isoforms	
	ApoA-I$_0$	ApoA-I$_{-1}$
ApoA-I tracer[a]	0.87	0.13
In vitro study[b]	0.84	0.16
In vivo study		
10 min	0.85	0.15
3 hr	0.85	0.15
12 hr	0.84	0.16
1 day	0.80	0.16
4 days	0.80	0.20
7 days	0.78	0.23
10 days	0.75	0.25
14 days	0.72	0.28

[a]Distribution of radioactivity after addition of ^{125}I-apoA-I$_0$ (100,000 cpm) to 200 μg of apoHDL in 8 M urea at the time of separation by isoelectrofocusing.

[b]Value from in vitro incubation of ^{125}I-apoA-I$_0$ (100,000 cpm) with 5 ml of normal plasma (anticoagulant, 0.1% EDTA) at 37°C for 30 min.

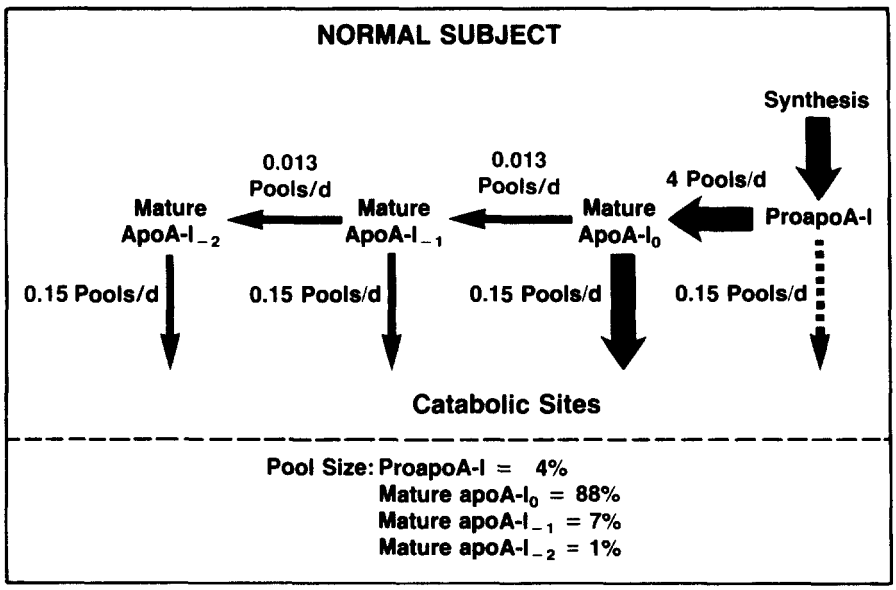

Fig. 5 Schematic overview of apoA-I isoprotein metabolism in man. ApoA-I is secreted from the liver and intestine as proapoA-I, which is rapidly and virtually quantitatively converted to mature apoA-I. Mature apoA-I is catabolized at a rate of 0.15 pools per day. The percentage of proapoA-I and mature apoA-I were determined by two-dimensional gel analysis of plasma (17). The rate constants in the schematic representation, which were derived from the experimental results, predict the isoform distribution of the apoA-I isoforms which was observed on two-dimensional electrophoresis.

with a residence time of approximately 6 days. There is slow conversion of the mature isoforms from apoA-I_0 to apoA-I_{-1} to apoA-I_{-2}. In normal man about 4% of apoA-I is present as proapoA-I with the remainder as the mature isoforms.

It is also of interest that apoA-II, the other major apolipoprotein of HDL, has recently been cloned and the complete nucleic acid sequence has been determined (30). ApoA-II, like apoA-I, is synthesized as preproapolipoprotein. However, in contrast to proapoA-I, proapoA-II contains two basic residues (Ala-Leu-Val-Arg-Arg-apoA-II) preceding the cleavage site for conversion to mature apoA-II (30, 31). Therefore, the sequences at the cleavage site of the propeptides for apoA-I and apoA-II are strikingly different suggesting separate enzyme systems being involved in the processing of the two proapolipoproteins. Recently we localized human apoA-I to the p11-q13 region of chromosome 11 (32), and apoA-II to the p21 → qter region of chromosome 1 (33), thus establishing that the two major apolipoproteins in HDL are on separate chromosomes in man.

The elucidation of the pathways for biosynthesis and processing of apoA-I and apoA-II and the identification of new apolipoprotein variants will undoubtedly provide new insights into apoA-I, apoA-II, and HDL metabolism, the modulation of plasma HDL levels, and the development of atherosclerosis.

Manuscript received 21 December 1983.

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