Apolipoprotein A-I isoforms in human lymph: effect of fat absorption

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Abstract The effect of fat feeding (100 g of cream) on the apoA-I isoproteins distribution has been analyzed by two-dimensional gel electrophoresis in the chylomicrons, VLDL, LDL, and HDL isolated from the thoracic duct lymph of patients undergoing lymph drainage for immunosuppression. Isoforms apoA-I_3 and apoA-I_4 are the most abundant apoA-I isoproteins in plasma lipoproteins as well as in lymph lipoproteins collected in the fasting state. Fat feeding, on the other hand, results in a marked change in the apoA-I isoform pattern in lymph chylomicrons and VLDL, with a significant increase in the relative concentration of the apoA-I_1 isoform. As a result the total concentration of this isoprotein in the lymph increased. The data indicate that fat feeding is associated with major changes in the distribution of the apoA-I isoforms in the lymph (d < 1.006 g/ml lipoproteins), which may be of significance in their plasma catabolism.—**Ghiselli, G., E. J. Schaefer, J. A. Light, and H. B. Brewer, Jr.** Apolipoprotein A-I isoforms in human lymph: effect of fat absorption. *J. Lipid Res.* 1983. **24:** 731–736.

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Apolipoprotein A-I (apoA-I) is a major protein constituent of plasma HDL and lymph lipoproteins in man and in other mammals (1, 2). ApoA-I is important in the metabolism of HDL, and it is known that modifications in the amino acid composition of apoA-I, such as that found in apoA-$I_{Tangier}$ (3), and apoA-I_{Milano} (4), produce profound effects on plasma HDL cholesterol levels, as well as on the concentrations of the other lipoprotein fractions and apolipoproteins. ApoA-I is polymorphic when analyzed by analytical two-dimensional electrophoresis (5–7). In this report the apoA-I isoforms have been denoted apoA-I_1 through apoA-I_5, and in plasma, apoA-I_3 and apoA-I_4 are the major forms.

Human apoA-I has been localized by immunochemical techniques in intestinal epithelial cells (8). It is estimated that approximately 50% of apoA-I total body synthesis takes place in the intestine in man and the rat (9–12). However, no data are available on the apoA-I isoform distribution in the human or animal lymph lipoproteins. This information may clarify the functional significance of the different apoA-I isoforms in the metabolism of plasma lipoproteins. In the present study, we have analyzed the pattern of apoA-I isoforms in plasma, and in four different lipoprotein fractions isolated from human thoracic duct lymph in the fasting and fat-fed state. The data indicate that fat feeding results in a significant increase in the relative proportion of the apoA-I_1 isoform in chylomicrons and VLDL.

MATERIALS AND METHODS

Lymph collection

Lymph was obtained by cannulation of the thoracic duct in subjects undergoing lymph drainage for purposes of immunosuppression before kidney transplantation. The patients maintained constant weight on an ad lib diet of 2000–3000 Kcal/day. Plasma triglycerides and cholesterol concentrations were within normal ranges for age- and sex-matched controls. The concentrations of both lipids did not vary significantly during the 24-hr collection.

The protocol for lymph drainage and collection has been described in a previous paper (9) and details are given in the legend for Table 1. Lymph collection in the fasting state was begun just before the morning meal. Post-absorptive lymph was collected for 6 hr, after a fat meal (100 g of cream). Lymph samples were collected in sterile bags containing 3 mM Na_2 EDTA, and kept on ice or refrigerated prior to lipoprotein isolation. The volume of drained lymph was measured for the calculation of lipid and apolipoprotein lymph transport rates (9).

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

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Lipoprotein isolation

For the determination of lipids and apoA-I lymph lipoprotein distribution, lymph was fractionated in a 40.3 Beckman rotor (Beckman Inc., Fullerton, CA) at densities 1.006, 1.063, and 1.210 g/ml (13). For chylomicron ($S_f > 400$) isolation, lymph was centrifuged in an SW 27 Beckman swinging bucket rotor for 115 min at 25,000 rpm at 4°C. During this procedure, the chylomicrons, concentrated at the top of the ultracentrifuge tube, were collected with a spatula. Before analysis, chylomicrons were dispersed in saline and washed twice by the same procedure. The infranate was utilized for the isolation of VLDL, LDL, and HDL as described. Plasma and lymph supernate (1.21 g/ml) were also obtained by ultracentrifugation. Lipoproteins were dialyzed against 0.08% ammonium bicarbonate before lyophilization and lipid extraction for apolipoprotein electrophoretic analysis.

Lipid and apolipoprotein quantitation

Total cholesterol and triglyceride concentrations in plasma, lymph, and different lipoprotein fractions were quantitated enzymatically using the Gilford 3500 autoanalyzer (Gilford Instrument Co., Oberlin, OH). The concentration of apoA-I in all samples was measured by radial immunodiffusion (14, 15). All samples were delipidated with methanol–ethyl ether 3:7 and the protein was resolubilized in 0.05 M sodium barbitol, 8 mM sodium azide, pH 8.3, for immunochemical assay (15). Apolipoprotein standards were obtained as previously described (16), and their concentrations were determined by amino acid analysis on a Beckman Model 121 automatic amino acid analyzer.

Two-dimensional gel electrophoretic analysis of apolipoproteins

The lipoprotein fractions were lyophilized and lipid was extracted with chloroform–methanol–diethyl ether 3:1:13 twice. The protein pellet was dissolved in 8.6 M urea, 5 mM Tris-HCl (pH 8.2) and the protein concentration was determined by the method of Bradford (17). Apolipoproteins were analyzed by two-dimensional gel electrophoresis as described by Anderson and Anderson (18). A pH gradient from 4 to 6 (Serva ampholines of Serva AB, Heidelberg, FRG), and a 7.5% polyacrylamide gel were utilized in the first dimension. Gels were electrophoresed at 250 volts for 16 hr. The upper buffer was 0.02 M NaOH, and the lower buffer was 0.01 M H_3PO_4. The second electrophoretic separation was performed on a polyacrylamide slab gel (15% acrylamide, 0.5% bisacrylamide, 0.1% SDS) in a BioRad 220 Dual Slab Cell (BioRad, Richmond, CA). Gels were stained with 0.1% Coomassie Blue R 250 in acetic acid–methanol–water 5:50:45, and destained in 5% methanol and 7.5% acetic acid until a clear background was obtained.

ApoA-I_1 isolation and identification

ApoA-I_1 was isolated for identification and chemical analysis by preparative isoelectrofocusing. Pooled chylomicrons and VLDL isolated from the lymph collected after fat feeding were utilized as starting material. The delipidated apolipoproteins were dissolved in 8.6 M urea, 5 mM Tris-HCl, pH 8.2, and separated by isoelectrofocusing over a pH range from 4 to 6 in a slab cell. The electrophoretic conditions were as described above for isoelectrofocusing in two-dimensional electrophoresis. After completion of the run, the protein bands were identified by staining a section of the gel cut from the side of the gel. The zone (1 cm wide) corresponding to apoA-I_1 isoprotein of the unstained gel was cut out, and loaded on the top of a polyacrylamide SDS gel (15% polyacrylamide. 0.4% bisacrylamide, 0.1% SDS), and electrophoresed to separate apoA-I_1 from ampholines and other protein contaminants. ApoA-I_1 was transferred from the SDS polyacrylamide gels onto an agarose slab gel (0.5% in 0.5 *M* Tris-HCl, pH 6.8) by electrophoresis and finally recovered by ultracentrifugation of the agarose in a 60 Ti Beckman rotor (30,000 rpm, 120 min). The protein was desalted by chromatography through a BioGel PD-6 (BioRad) column (50 × 1.2 cm) equilibrated with 0.08% ammonium bicarbonate.

The identity and the purity of the isolated protein were analyzed by Ouchterlony immunodiffusion (19) against antibodies to apoA-I, apoA-IV, apoC-II, apoE, and apoH. Basic electrophoresis in 7.5% polyacrylamide gel containing 8 M urea was performed as described by Reisfeld and Small (20). The apparent molecular weight of apoA-I_1 was determined by SDS-polyacrylamide slab gel electrophoresis (21). Amino acid analyses were performed on a Beckman Model 121 amino acid analyzer equipped with a 126 Data Processor, and a 6-mm column assembly. Acid hydrolyses were performed in 6 M HCl (Pierce Chemical Co.) at 110°C with 2-mercaptoethanol (1:2000 v/v).

RESULTS

Effect of fat feeding on the lipoprotein distribution and transport rate of lipid and apoA-I in lymph

Mean (±SEM) triglyceride and cholesterol concentrations in three samples of lymph from fasted subjects, each collected 2 hr apart, starting 6 hr before fat feeding, were 268 ± 57 mg/dl and 45 ± 4 mg/dl, respectively. The lymph apoA-I concentration was 31.49

± 1.51 mg/dl, and the majority of apoA-I was associated with HDL (see **Table 1**). Fat feeding resulted in a sharp increase in the lymph triglyceride concentration (3828 ± 155 mg/dl) with no increase in the cholesterol level (45 ± 3 mg/dl). The concentration of apoA-I in the lymph remained virtually unchanged (27.03 ± 1.43 mg/dl); however, the apoA-I content of chylomicrons and VLDL increased 27-fold over the preabsorptive level (4.40 ± 0.72 mg/dl vs. 0.16 ± 0.01 mg/dl). The lymph transport rates of triglyceride, cholesterol, and apoA-I in the different lipoprotein fractions are shown in Table 1.

ApoA-I isoform distribution in lymph lipoproteins

ApoA-I, either in plasma or in lymph, is composed of a number of isoforms. The same isoproteins detected in plasma are also present in the lymph from fasted or fat-fed individuals. **Fig. 1** illustrates the apoA-I polymorphism in plasma (panel A) and lymph (panel B) following the analysis of the 1.21 g/ml supernate by two-dimensional electrophoresis. The apoA-I isoproteins have been designated by numbers as indicated in panel A. The apoA-I isoform pattern was identical in plasma or fasted lymph, but was significantly different in the lymph from fat-fed subjects, due to the relative increase of apoA-I_1, a minor isoprotein in plasma or fasted lymph.

To further evaluate the significance of these findings, the apoA-I isoform pattern was analyzed in chylomicrons, VLDL, LDL, and HDL isolated from lymph samples from fasted and fat-fed subjects (**Fig. 2**). In the fasted lymph, the relative concentration of the apoA-I isoforms remained very similar throughout the lipoprotein density spectrum. On the other hand, fat absorption resulted in a dramatic change in the quantitative distribution of the isoforms of lymph chylomicrons and VLDL, with a marked increase in concentration of apoA-I_1. The apoA-I isoform pattern in LDL and HDL, however, were not affected by fat feeding. Similar results were obtained when the lymph from a second subject was analyzed and the effect of fat feeding was examined under the same protocol.

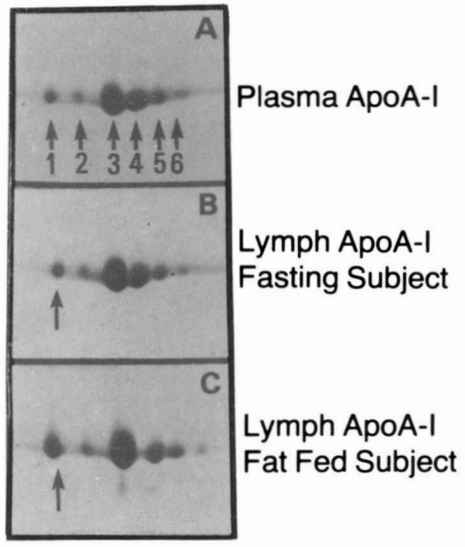

Fig. 1. Two-dimensional gel electrophoretograms of apoA-I from the d < 1.21 g/ml of plasma (panel A) and fasted and fat-fed lymph lipoproteins (panels B and C, respectively). Lymph samples for the analyses were collected for approximately 2 hr before and in the 4 hr following the fat meal. Only the area of the gel corresponding to apoA-I is presented. The different apoA-I isoforms were similar to those previously reported (5). The arrow indicates the position of apoA-I_1 in panels B and C. Thirty ug of apolipoproteins was loaded per gel.

ApoA-I_1 identification and analysis

ApoA-I_1 isolated by preparative isoelectrofocusing was analyzed for purity by a variety of electrophoretic and immunological techniques. The apoA-I isoform

TABLE 1. Lipids and apoA-I transport rates (mg × hr^{-1})[a] by lymph lipoproteins[b]

		Lymph Lipoprotein Fractions		
		d < 1.006 g/ml	d 1.006–1.063 g/ml	d 1.063–1.210 g/ml
Triglycerides (mg/dl)	Fasting	740 ± 234	52 ± 9	49 ± 9
	Fat feeding	3611 ± 529	85 ± 13	59 ± 9
Cholesterol (mg/dl)	Fasting	42 ± 8	68 ± 13	21 ± 1
	Fat feeding	100 ± 18	39 ± 4	22 ± 14
ApoA-I (mg/dl)	Fasting	0.61 ± 0.07	<0.10	91.94 ± 9.93
	Fat feeding	16.63 ± 3.02	1.26 ± 0.43	83.79 ± 3.34

[a] Mean ± SEM; n = 3.

[b] Lymph in the fasting condition was collected from 2:00 AM to 8:00 AM in sterile bags containing 3 mM Na_2 EDTA. Collection of lymph after the fat meal was performed from 8:00 AM to 2:00 PM. The values, designated under Fat feeding were determined in lymph samples obtained during the last 4 hr of the study. Transport rates have been obtained by multiplication of the lipids or apoA-I lymph concentrations (as mg/dl) by the lymph flux (as dl/hr) (see Ref. 9).

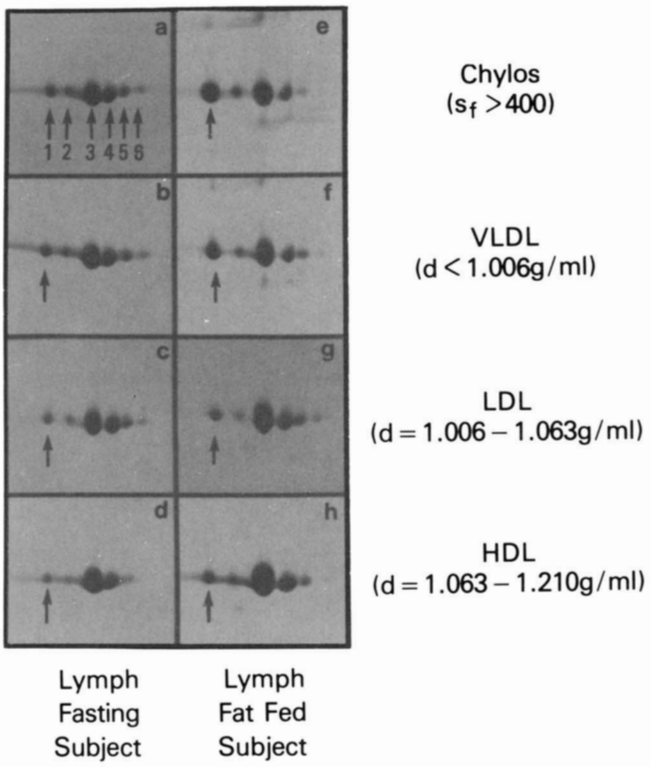

Fig. 2. Two-dimensional gel electrophoretograms of apoA-I in lymph lipoproteins. Panels a to d illustrate the apoA-I isoforms in lipoproteins obtained from fasted lymph. The results from lipoproteins isolated from lymph from fat-fed subjects are presented in panels e to h (see legend to Fig. 1). Only the area of the gel corresponding to apoA-I is shown. The apoA-I isoforms in panel a are identified by numbers. The arrow in the other panels indicates the position of apoA-I_1.

pattern of the chylomicrons plus VLDL (d < 1.006 g/ml) fraction used for the isolation of apoA-I_1 and the two-dimensional gel electrophoretograms of purified apoA-I_1 are illustrated in **Fig. 3.** Isolated apoA-I_1 migrated as a major single electrophoretic isoform with two additional minor components. The two minor components were present in three separate isolated preparations of apoA-I_1 obtained by preparative isoelectrofocusing, and probably represent minor variants of the major isoform. Their concentration was noted to increase during storage at −20°C for 2 to 4 weeks. The purity of apoA-I_1 was also analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis (**Fig. 4**). ApoA-I_1 migrated as a single electrophoretic band (15% acrylamide), with a similar apparent molecular weight as total apoA-I isolated by column chromatography from plasma HDL. ApoA-I_1 had a more basic isoelectric point and migrated at a slower rate when compared to the major apoA-I isoforms in basic polyacrylamide gel electrophoresis in 8 M urea.

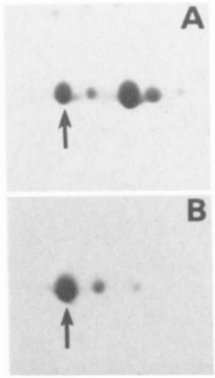

Fig. 3. Two-dimensional gel electrophoretograms of apoA-I from lymph lipoproteins from fat-fed subjects (d < .1006 g/ml) (panel A) utilized as starting material for apoA-I_1 isolation by preparative isoelectrofocusing, and of isolated apoA-I_1 (panel B).

Purified apoA-I_1 formed an immunoprecipitin line with an anti-apoA-I antibody, and a line of identity with purified total apoA-I as shown in **Fig. 5.** Isolated apoA-I_1 did not react with antibodies prepared against apoA-II, apoA-IV, apoC-II, apoB, apoE, and apoH.

Amino acid analysis of purified apoA-I_1 was similar to the known amino acid composition of apoA-I; (mol percent) asp, 8.85; thr, 4.22; ser, 6.14, glu, 18.77; pro, 4.56, gly, 4.21, ala, 8.23, val, 5.23; met, 1.06; leu, 15.42, tyr, 3.01, phe, 2.65, his, 2.04, lys, 8.91, and arg, 6.68.

DISCUSSION

The small intestine is an active site of synthesis and secretion of a number of plasma apolipoproteins. Rat small intestine has been reported to synthesize large

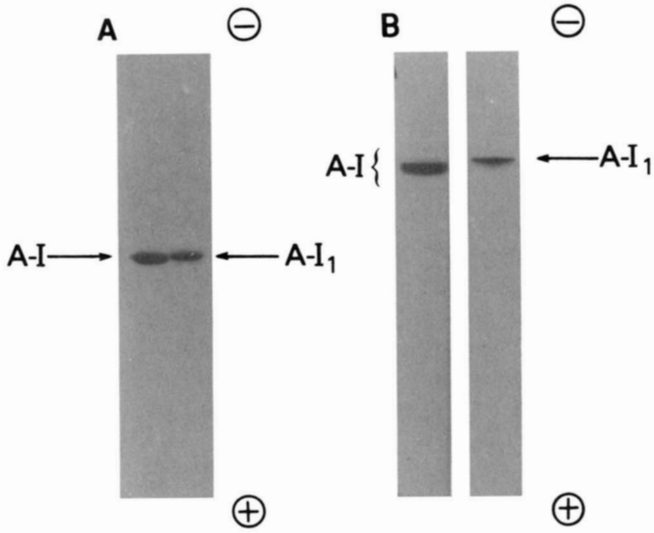

Fig. 4. SDS polyacrylamide gel electrophoresis (panel A) and basic polyacrylamide gel electrophoresis in 8 M urea (panel B) of total apoA-I (electrophoretogram on the left in each panel) and apoA-I_1 isolated by preparative isoelectrofocusing (electrophoretogram on the right in each panel).

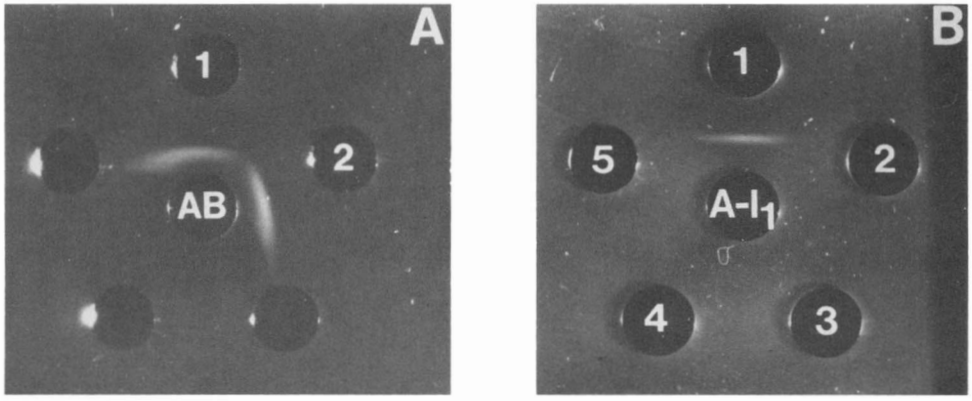

Fig. 5. Ouchterlony immunodiffusion of apoA-I_1. In panel A the central well contains anti-apoA-I antibody and the outer wells contain total apoA-I (position 1) and apoA-I_1 (position 2). In panel B the central well contains apoA-I_1 and the other wells contain anti-apolipoprotein antibodies in the following order: position 1, anti-apoA-I; position 2, anti-apoA-IV; position 3, anti-apoC-II; position 4, anti-apoE, and position 5, anti-apoH.

quantities of apoA-I, apoA-IV, and apoB, but only small amounts of the C and E apolipoproteins (10). Human apoA-I has been localized in the intestinal epithelial cells by immunological techniques, and synthesis has been demonstrated by isotopic incorporation (5–9, 22). Intestinal chylomicron apoA-I in man (23) and rats (24) has been shown to be rapidly transferred to HDL after the chylomicrons reach the blood stream.

ApoA-I is polymorphic in plasma and is composed of a series of isoproteins of similar molecular weights (5, 25, 26). The structural differences between the various isoforms have not been reported. In man, the major plasma apoA-I isoproteins, apoA-I_3 and apoA-I_4, have indistinguishable amino acid composition (25–27) and activate lecithin cholesterol acyltransferase (LCAT) to the same extent (26).

Utilizing organ culture of either human fetal or adult intestine, or fetal liver, Zannis et al. (5–7) have shown that the newly synthesized apoA-I consists primarily of the apoA-I isoproteins, which have a more basic isoelectric point than the two major apoA-I isoproteins in plasma, apoA-I_3 and apoA-I_4. These observations may be interpreted as indicating that apoA-I_1 and apoA-I_2 are converted to apoA-I_3 and apoA-I_4 rapidly after secretion since the latter two isoforms are the predominant apoA-I isoforms in plasma.

A study conducted in our laboratory on HDL isolated from normal and dyslipidemic subjects with different forms of hyperlipoproteinemia indicates that the ratio of the different apoA-I isoproteins is fairly constant in plasma (data not shown). The apoA-I isoform pattern in human thoracic duct lymph has not been reported previously. The extravascular pool of apoA-I appears to be quantitatively important in man, and it is likely that the lymph is an important physiological space (9). It has been determined in man that the mass of apoA-I transported by thoracic duct lymph ranges between 1,000 to 2,400 mg/day (9). In this study, we have analyzed the apoA-I isoprotein pattern in different lipoprotein fractions isolated from thoracic duct lymph, and investigated the effect of fat absorption on the isoprotein distribution. The results demonstrate that lymph lipoproteins either isolated from lymph from fasted or fat-fed subjects exhibit an apoA-I polymorphism composed of the same series of isoproteins observed in plasma. Lymph lipoproteins including chylomicrons, isolated in the fasting state, have an isoform pattern virtually identical to that observed for plasma apoA-I. Fat feeding, on the other hand, increases the relative concentration of the apoA-I_1 isoform in chylomicrons and VLDL and the mass of this isoprotein in the lymph is increased (see Fig. 1).

The site of origin of apoA-I_1 cannot be clearly established from these experiments. However, it is likely that the increase in apoA-I_1 during active fat absorption represents direct intestinal biosynthesis, and that the chylomicrons and VLDL are secreted into the lymph with an increased content of apoA-I_1. Alternatively, apoA-I_1 may have a high affinity for triglyceride-rich lipoprotein particles, and an increase in concentration of these lipoproteins during fat absorption could result in an increase in apoA-I_1 content in these lipoproteins by transfer from a pre-existing pool of apoA-I_1. In either case, these results provide the first direct evidence that the pattern of apoA-I isoforms in plasma or lymph may be modulated by diet.

Note Added in Proof: We have recently shown by direct sequence analysis that apoA-I_1 is proapoA-I, and contains six amino acids, arg-his-phe-trp-gln-gln, attached to the amino-terminal end of mature apoA-I. In addition, metabolic studies showed that radiolabeled proapoA-I was rapidly converted to mature

apoA-I (apoA-I_3 and apoA-I_4) in normal humans (Ghiselli, G., E. J. Schaefer, S. Law, J. A. Light, and H. B. Brewer, Jr. 1983. *Clin. Res.* **31:** 500A).

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