Apolipoprotein E metabolism in normolipoproteinemic human subjects

Richard E. Gregg, Loren A. Zech, Ernst J. Schaefer,[1] and H. Bryan Brewer, Jr.

Molecular Disease Branch, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bethesda, MD 20205

Abstract Human apolipoprotein E (apoE) is a constituent of plasma very low density and high density lipoproteins and is important in modulating the catabolism of remnants of triglyceride-rich lipoproteins. There are three common isoforms of apoE, designated apoE-2, E-3, and E-4, which are coded by three separate alleles (ε2, ε3, and ε4) at a single genetic locus and inherited in the population in a co-dominant fashion. ApoE-3 is the predominant apoE isoform in the normolipidemic population, and ε3 has been proposed to be the normal allele. ApoE-3 metabolism was studied in nine normolipidemic subjects homozygous for the ε3 allele. In these subjects, the plasma apoE-3 concentration was 4.8 ± 1.2 mg/dl (mean ± SD), the plasma apoE-3 residence time was 0.73 ± 0.18 days, and the plasma apoE-3 production rate was 3.4 ± 1.5 mg/kg-day. The apoE in males, when compared to females, tended to have a shorter residence time (0.63 ± 0.15 days versus 0.83 ± 0.16), a higher production rate (4.20 ± 1.73 mg/kg-days versus 2.60 ± 0.78), but a similar plasma concentration (5.1 ± 1.5 mg/dl versus 4.5 ± 0.8). ApoE-3 had a more rapid catabolism from plasma than other apolipoproteins previously studied (apolipoproteins A-I, A-II, A-IV, B-100, C-II, and C-III) except for apolipoprotein B-48. The catabolism of apoE-3 in the individual lipoprotein subfractions was also examined and apoE was shown to be catabolized most rapidly from the VLDL and slowest from the HDL. The results of the kinetic analysis of apoE metabolism are consistent with apoE being important in the catabolism of triglyceride-rich lipoproteins and with HDL serving as a reservoir for apoE to reassociate with newly secreted triglyceride-rich lipoproteins.—**Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr.** Apolipoprotein E metabolism in normolipoproteinemic human subjects. *J. Lipid Res.* 1984. **25:** 1167–1176.

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Apolipoprotein E (apoE), a glycoprotein of molecular weight 34,000 daltons, is associated predominantly with VLDL and HDL in human plasma (1, 2). Human apoE is a polymorphic protein (3) having three major isoforms separable by isoelectrofocusing (apoE-2, apoE-3, and apoE-4) (3, 4). The complete primary amino acid sequence of apoE-2 is known (5) and the partial primary amino acid sequences of apoE-3 and an apoE-2 variant have been reported (5, 6). Each of the known polymorphic forms of apoE is coded by a separate allele and these alleles are inherited in a co-dominant fashion at a single genetic locus[2] (4). ApoE-3 is the most common isoform in the population (3, 4, 7), and is virtually always associated with normolipidemia; as a result, apoE-3 has been proposed to be the normal allele. ApoE-1, apoE-2, and apoE deficiency are associated with type III hyperlipoproteinemia (3, 4, 8, 9). We have also observed an increased prevalence of apoE-4 in patients with severe type V hyperlipoproteinemia (7).

ApoE has been demonstrated to bind in vitro to the high affinity LDL or apoB,E receptor on fibroblasts and to a distinct hepatocyte apoE receptor (10). It has been proposed that apoE is the recognition site on chylomicrons for the receptor-mediated removal of chylomicron remnants by the liver (10, 11). In addition, apoE has been reported to both inhibit (12, 13) and activate (13, 14) lipoprotein lipase. Whether either of these functions is the major physiological function of apoE is as yet unknown.

To gain further insights into the function and metabolism of apoE, we have initiated a systematic in vivo analysis of apoE metabolism in humans. We report here the results of our analysis of the metabolism of apoE-3 in normolipidemic subjects with an E3/3 phenotype.

Abbreviations: apoE, apolipoprotein E; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

[1] Current address: Endocrinology Division, New England Medical Center, and Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111.

[2] There are three major isoforms of apoE separable by IEF-PAGE. These isoforms are designated apoE-2, apoE-3, and apoE-4, and are coded by three common alleles, ε2, ε3, and ε4. An individual may be either homozygous for one or heterozygous for two apoE alleles, which are codified as ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, and ε3/ε4. When analyzed by IEF-PAGE, homozygous genotypes are identified as apoE phenotypes E2/2, E3/3, and E4/4, while heterozygous genotypes are manifested as apoE phenotypes E3/2, E4/2, and E4/3.

METHODS

Study subjects

The nine subjects studied were healthy young adult males (n = 5) and females (n = 4), ages 20–23 years, who were hospitalized in the Clinical Center of the National Institutes of Health (**Table 1**). All subjects had normal fasting glucose levels, as well as normal thyroid, liver, and renal function. The subjects were not on any medication and had no serious illness. All subjects gave informed consent and the study protocol was approved by the Human Use Research Committee of the National Heart, Lung, and Blood Institute.

Isolation of apoE

ApoE was isolated and characterized as previously described (15). Briefly, VLDL was obtained by ultracentrifugation (d < 1.006 g/ml) and delipidated with chloroform–methanol 3:1(v/v). ApoE was separated from the other apolipoproteins by heparin affinity and Sephacryl S-200 gel permeation chromatography. Purified apoE was a single electrophoretic band on SDS (15% acrylamide) and urea (pH 8.4) PAGE (15).

Iodination of apoE and VLDL

ApoE was radioiodinated by a modification of the iodine monochloride method (16, 17). One hundred μg of apoE was lyophilized and redissolved in 50 μl of buffer containing 6 M guanidine-HCl and 1 M glycine (pH 8.5) in a small conical test tube. Five mCi of carrier-free $Na^{125}I$ or $Na^{131}I$ (New England Nuclear, Boston, MA) was added and the sample was vortexed. Fifteen μl of 33 mM iodine monochloride in 150 mM NaCl was added slowly over 10–15 sec while vortexing the sample. ApoE was iodinated with an efficiency of 35–50% with incorporation of approximately 0.5 mole of iodine per mole of apoE. Radiolabeled apoE was incubated with 75 ml of plasma from a normal fasting subject for 30 min at 37°C, the density of the plasma was adjusted to 1.21 g/ml with solid KBr, and the sample was centrifuged in a Beckman 60Ti rotor (Beckman Instrument, Inc.) for 2.2×10^8 *g*-min. The d 1.21 g/ml supernatant was separated by tube slicing and dialyzed against 100 volumes × 5 of a sterile solution of 150 mM NaCl, 0.1 M Tris-HCl (pH 7.4), and 0.01% EDTA. Human serum albumin (American Red Cross Services, Washington, DC) was added to a final concentration of 1% (wt/vol) and the sample was sterilized by filtration through a 0.22-micron Millipore filter. Each preparation was tested for pyrogens prior to injection into the study subjects.

VLDL was isolated and iodinated by a modification of the method described by Bilheimer, Eisenberg, and Levy (18). Plasma from fasting subjects (160 ml) was centrifuged at plasma density for 1.12×10^8 *g*-min at 4°C. VLDL, the d 1.006 g/ml supernate, was obtained by tube slicing and washed once by repeat ultracentrifugation. One ml of VLDL (4.0 mg/ml of protein) was added to 1 ml of 1.0 M glycine (pH 10.0). Five mCi of carrier-free $Na^{125}I$, (New England Nuclear, Boston, MA) was added to the reaction mixture followed by the slow addition of 240 μl of 33 mM ICl with gentle mixing. The quantity of ICl added was calculated so that approximately 0.5 mol of iodine was bound per mol of VLDL protein, assuming a mean molecular weight for the protein of 250,000 daltons. The ^{125}I-labeled VLDL was then dialyzed and prepared for injection as described above for radiolabeled apoE. The efficiency of iodination was 10%, and 14% of the covalently attached iodine was bound to lipid.

Study protocol

The study subjects were placed on a defined isoweight diet containing 42% carbohydrate, 42% fat, 16% protein, 200 mg of cholesterol per 1000 Kcal, and a polyunsat-

TABLE 1. Characteristics of study subjects[a]

	A	B	C	D	E	F	G	H	I
Sex	F	F	F	F	M	M	M	M	M
Height (cm)	163	168	165	173	185	185	183	183	180
Weight (kg)	57.2	55.0	56.1	57.2	90.8	84.7	73.6	75	76.5
Age (yr)	22	20	20	22	21	21	20	23	20
Cholesterol[b]	147	185	173	142	125	159	143	183	133
Triglycerides	56	58	75	55	74	88	66	54	80
VLDL-cholesterol	13	10	10	8	5	5	12	10	13
LDL-cholesterol	75	102	93	78	92	112	64	79	87
HDL-cholesterol	59	73	70	56	28	42	67	94	33
VLDL-cholesterol/ triglyceride ratio	0.23	0.17	0.13	0.15	0.07	0.06	0.18	0.19	0.16
ApoE	3.5	4.3	5.3	4.5	3.1	5.9	6.6	5.8	3.2

[a] All subjects had an E3/3 apoE phenotype.
[b] All lipid and apolipoprotein values are expressed as mg/dl.

urated to saturated fat ratio of 0.1 to 0.3 for 10 days before beginning the apoE metabolic studies. The diet was changed to a liquid formula of the same nutrient composition given every 6 hr 3 days before the apoE injection, and continued for the duration of the study. Three days prior to study, the subjects were also started on potassium iodide (1200 mg per day) and ferrous gluconate (900 mg per day) in divided doses.

Subjects were injected intravenously with up to 25 μCi of ^{131}I-radiolabeled apoE, 100 μCi of ^{125}I-radiolabeled apoE, or 16 μCi of radiolabeled VLDL approximately 5.5 hr after their previous meal. Blood samples were obtained at 10 min and approximately 30 min later, just before their next meal. Additional blood samples were drawn at 6 hr, 12 hr, 18 hr, 24 hr, 36 hr, 48 hr, and then daily through day 7. All samples except the first one were drawn just prior to a meal. i.e., after a 6-hr fast.

Blood samples of 20 ml were drawn into tubes containing EDTA at a final concentration of 0.1%. The blood was placed on ice and plasma was separated by low speed centrifugation (2000 rpm, 30 min) in a refrigerated centrifuge (4°C). Sodium azide and aprotinin (Boehringer Mannheim, West Germany) were added to plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. One ml of plasma was frozen at −20°C for apoE quantitation. The plasma lipoproteins were isolated by ultracentrifugation (19) and 1 ml of each subfraction was frozen (−20°C) for apoE quantitation. In the study where radiolabeled VLDL was employed, the quantity of radioactivity associated with apoE was determined in VLDL delipidated with chloroform–methanol 3:1 (v/v) (20) followed by SDS-PAGE (8% polyacrylamide and 2% bisacrylamide) (21). The area of the gel containing apoE was cut out, and the radioactivity was quantitated. The radioactivities in plasma, the lipoprotein subfractions, and gel slices were quantitated in a Packard Autogamma 5260 gamma counter (Packard Instrument Co., Morton Grove, IL).

Analytical methods

ApoE phenotypes were ascertained in each subject studied by analytical isoelectrofocusing (7). Plasma cholesterol and triglycerides were quantitated on a Gilford System 3500 enzymic analyzer. HDL cholesterol was determined in plasma following dextran sulfate precipitation (22). All other lipid and lipoprotein analyses were performed by the methods of the Lipid Research Clinics (23). Plasma lipoproteins were fractionated by gel permeation chromatography using a Sepharose CL-6B column (1.2 × 95 cm) with an eluting buffer of 0.15 M NaCl, 0.1 M Tris-HCl (pH 7.4), and 0.01% EDTA. The flow rate was 18 ml/hr and 1.1-ml fractions were collected. ApoE was quantitated by a double antibody radioimmunoassay (15). The residence time (1/fractional catabolic rate) was determined from the area under the plasma decay curve by a multiexponential computer curve-fitting technique (24). The production rate was calculated from the following formula: production rate = (apoE concentration × plasma volume)/(residence time × weight) with the plasma volume being determined by dividing the total quantity of radioactivity injected by the radioactivity per unit volume determined in the sample obtained 10 min after injection. Intergroup comparisons were performed using the Student's *t* test.

RESULTS

Characterization of radiolabeled apoE

The effect of radioiodination on the electrophoretic properties of apoE was determined by the analysis of radiolabled apoE-3 combined with 5 μg of purified apoE-3. Greater than 90% of the radioactivity comigrated with the apoE band on SDS-PAGE, and the radioactivity associated with the apoE isoforms was proportional to the relative quantity of each isoform on isoelectrofocusing.

The distribution of apoE among plasma lipoproteins is determined by the laws of mass action. The effect of iodination on the distribution of apoE-3 among plasma lipoproteins was determined in vitro and in vivo following ultracentrifugation, and by gel permeation chromatography. A similar distribution of radiolabeled apoE and unlabeled apoE with constant specific activities among the plasma lipoproteins would indicate that iodination had no significant effect on the physicochemical and lipid binding properties of apoE.

The distribution of radiolabeled apoE-3 was initially determined in vitro by incubation of 0.2 μCi of ^{131}I-labeled apoE with 5 ml of plasma from normal subjects for 30 min at 37°C. The plasma lipoproteins were separated by ultracentrifugation, and the apoE radioactivity and apoE mass were quantitated. ApoE was present primarily within VLDL and HDL (**Table 2**). The specific activities of each subfraction were determined and normalized to one. The normalized specific activities did not significantly vary from one except for IDL, and it comprised less than 5% of the total radioactivity in plasma (**Table 3**).

The in vivo distribution of radiolabeled apoE was determined from plasma obtained 10 min following the injection of radiolabeled apoE into normal subjects. The plasma was fractionated by ultracentrifugation, and the apoE radioactivity and mass were quantitated in the individual lipoprotein fractions. The distribution of radiolabeled apoE within the plasma lipoproteins in the in vivo study was virtually identical to the distribution

TABLE 2. Distribution of radiolabeled apoE-3 in lipoprotein subfractions

	VLDL	IDL	LDL	IDL + LDL	HDL_{2b}	HDL_{2a+3}	Total HDL	1.21 g/ml Infranate
				percent ± SD				
In vitro incubation[a]	29.9 ± 3.2 (n = 6)	4.2 ± 1.0 (n = 6)	10.8 ± 0.8 (n = 6)	15.0 ± 1.4 (n = 6)	21.2 ± 3.1 (n = 6)	16.7 ± 4.0 (n = 6)	37.9 ± 4.0 (n = 6)	17.3 ± 0.6 (n = 6)
Ten-minute in vivo sample[b]	23.1 ± 6.0 (n = 12)	4.5 ± 0.7 (n = 8)	11.9 ± 2.1 (n = 8)	14.6 ± 3.3 (n = 12)	24.9 ± 3.5 (n = 6)	17.3 ± 1.8 (n = 6)	41.4 ± 6.5 (n = 12)	20.9 ± 3.6 (n = 12)

[a] Radiolabeled apoE-3 (0.2 μCi) was incubated with 5 ml of plasma at 37°C for 30 min, followed by fractionation of the plasma lipoproteins by ultracentrifugation.

[b] Ten minutes following the injection of radiolabeled apoE-3 into normal subjects, 5 ml of plasma was obtained and the lipoproteins were fractionated by ultracentrifugation.

observed in the in vitro study (Table 2). The plasma specific activities in the studies ranged from 222–971 cpm/μg of apoE, and none of the normalized specific activities differed from one except for IDL (**Table 4**).

The in vivo plasma kinetics of apoE radiolabeled as an apolipoprotein and apoE radiolabeled as a constituent of VLDL were analyzed. ApoE and VLDL were isolated from the same subject and radioiodinated. ApoE was radioiodinated with ^{131}I, incubated with the subjects' plasma at 37°C for 30 min, and the VLDL was isolated by ultracentrifugation. VLDL isolated from a separate sample of plasma was radiolabeled with ^{125}I. The subject was injected with 22.5 μCi of ^{131}I-labeled apoE-VLDL and 16.0 μCi of ^{125}I-labeled VLDL. The plasma decay of apoE was quantitated. Since all of the apolipoproteins on the injected VLDL were radiolabeled, it was necessary to isolate apoE to determine its rate of decay. At each sampling time, VLDL from 5 ml of plasma was isolated by ultracentrifugation, delipidated, and apoE was isolated by SDS-PAGE. The radioactivities associated with ^{125}I-labeled apoE and ^{131}I-labeled apoE were determined in the apoE band cut from the gel, and the results are presented in **Fig. 1.** The ^{131}I-labeled apoE labeled as the apolipoprotein decayed at virtually the same rate as ^{125}I-labeled apoE labeled as VLDL, i.e., there was no difference in the rate of catabolism of apoE labeled on a lipoprotein or labeled as an apolipoprotein and then reassociated with lipoproteins.

The distribution of radiolabeled apoE was also determined following the separation of plasma by gel permeation chromatography. Plasma for analysis was obtained 10 min following injection of 75 μCi of ^{125}I-labeled apoE-3 into a normal subject. The chromatographic profile of radioactivity and apoE concentration as determined by radioimmunoassay were very similar (**Fig. 2**). Within VLDL the apoE radioactivity and apoE concentration peaks chromatographed with the same K_d as the VLDL triglyceride peak; however, the apoE within HDL preceded the major HDL cholesterol peak, consistent with the concept that the major portion of apoE in HDL resides on the larger particles within this density range (1, 2). The specific activities of apoE within VLDL (663 cpm/μg), LDL (671 cpm/μg), and HDL (684 cpm/μg) were similar.

The distribution of apoE among the plasma lipoproteins was different when analyzed by ultracentrifugation and gel permeation chromatography, as has been previously found (1, 2). The majority of apoE-3 was associated with plasma lipoproteins by column chromatography; however, a significant fraction (21%) was present in the d 1.21 g/ml infranate following ultracentrifugation, indicating a significant alteration in apoE distribution as a result of ultracentrifugation.

The combined results from these studies indicate that radioiodination of apoE did not significantly alter the properties of apoE. Similar apoE specific activities were observed in the isolated lipoproteins regardless of the technique used to separate the fractions. Based on these results it was concluded that radiolabeled apoE was an effective tracer, and all subsequent studies were performed with radiolabeled apoE in which the apoE was labeled as an apolipoprotein and then reassociated with plasma lipoproteins.

Radiolabeled apoE kinetic studies

Twelve apoE-3 kinetic studies were performed in nine normal individuals with an E 3/3 phenotype (two females and one male were studied twice). ApoE was radiolabeled, associated with plasma lipoproteins, injected into the subjects, and samples of plasma were obtained at timed intervals.

The distribution of apoE mass was determined in the lipoprotein subfractions 10 min following the injection of radiolabeled apoE (Table 4). In the male subjects, 39 ± 17% (±SD) of the apoE mass in the lipoprotein subfractions was in VLDL with 19 ± 6% in HDL; in females, VLDL contained 14 ± 1%, and HDL 37 ± 10%.

TABLE 3. ApoE concentration and normalized specific activities in lipoprotein subfractions following 30 minutes in vitro incubation with radiolabeled apoE-3

	Plasma	VLDL	IDL	LDL	IDL + LDL	HDL_{2b}	HDL_{2a+3}	Total HDL	1.21 g/ml Infranate
ApoE concentration[a] (mg/dl) (n = 6)	5.19 ± 0.38[b]	1.22 ± 0.18	0.12 ± 0.02	0.50 ± 0.08	0.61 ± 0.06	0.80 ± 0.06	0.45 ± 0.03	1.25 ± 0.15	0.48 ± 0.06
ApoE specific activity[c] (n = 6)	1	1.18 ± 0.15	1.60 ± 0.18	1.02 ± 0.13	1.09 ± 0.11	0.86 ± 0.08	1.02 ± 0.10	0.92 ± 0.09	1.07 ± 0.08

[a] The sum of the apoE concentrations within the subfractions is less than the plasma apoE concentration due to loss occurring during sample handling and repeated sequential ultracentrifugation.
[b] Mean ± SEM.
[c] The specific activity is normalized to the plasma specific activity.

TABLE 4. ApoE concentration and normalized specific activities in lipoprotein subfractions 10 minutes following the injection of radiolabeled apoE-3

	Plasma (n = 12)	VLDL (n = 12)	IDL (n = 8)	LDL (n = 8)	IDL + LDL (n = 12)	HDL_{2b} (n = 6)	HDL_{2a+3} (n = 6)	Total HDL (n = 12)	1.21 g/ml Infranate (n = 12)
ApoE concentration[a] (mg/dl)	4.83 ± 0.35[b]	1.05 ± 0.16	0.10 ± 0.01	0.44 ± 0.07	0.50 ± 0.05	0.88 ± 0.06	0.44 ± 0.02	1.12 ± 0.12	0.55 ± 0.06
ApoE specific activity[c]	1	1.06 ± 0.13	2.06 ± 0.20	1.28 ± 0.17	1.30 ± 0.13	0.92 ± 0.08	1.08 ± 0.10	1.06 ± 0.10	1.13 ± 0.08

[a] The sum of the apoE concentration within the subfractions is less than the plasma apoE concentration due to loss occurring during sample handling and repeated sequential ultracentrifugation.
[b] Mean ± SEM.
[c] The specific activity is normalized to the plasma specific activity. The actual plasma specific activities varied from 222 to 971 cpm/μg apoE in the individual studies.

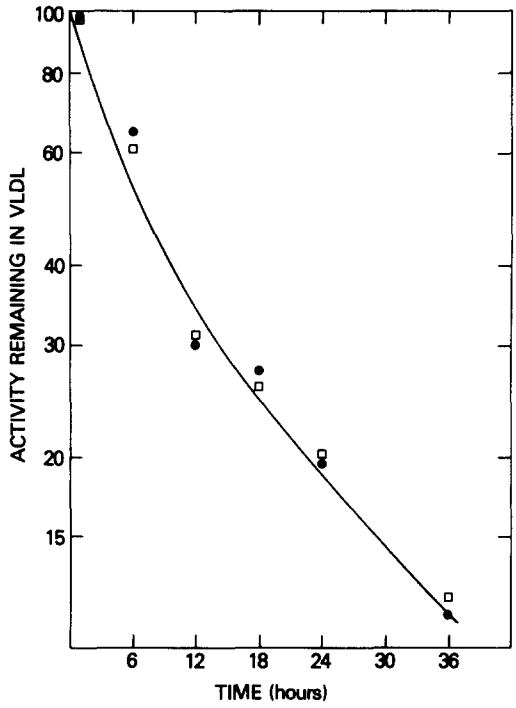

Fig. 1. The decay of apoE from plasma with apoE radioiodinated as an apolipoprotein and as a lipoprotein. ApoE was radioiodinated with ^{131}I, reassociated with lipoproteins, and VLDL was isolated by ultracentrifugation. In a separate plasma sample, VLDL was obtained by ultracentrifugation and radioiodinated with ^{125}I. Both VLDL preparations were injected intravascularly, plasma was obtained, VLDL was isolated by ultracentrifugation, and apoE was isolated by SDS-PAGE. The catabolism of apoE labeled as the apolipoprotein is indicated by □, while the catabolism of the lipoprotein-labeled apoE is indicated by ●. The values have been normalized with the amount of radioactivity at the first time point being 100%.

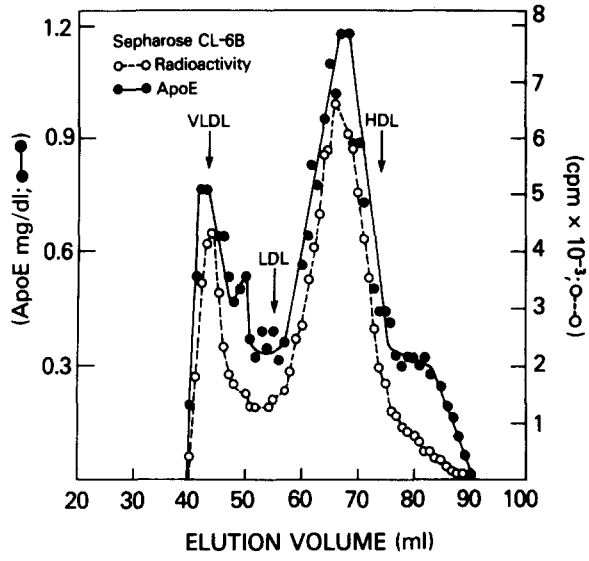

Fig. 2. The profile of apoE mass (● —— ●) and apoE radioactivity (O - - - O) in normal human plasma separated by 6% agarose column chromatography. Ten min following the injection of radioiodinated apoE into subject H, 5 ml of plasma was obtained and fractionated by column chromatography on a 1.2 × 95 cm column of Sepharose CL-6B (6% agarose). The eluting buffer was 0.15 M NaCl, 0.1 M Tris-HCl (pH 7.4), 0.01% EDTA. The flow rate was 18 ml/hr and 1.1-ml fractions were collected. The arrows for VLDL, LDL, and HDL indicate the relative K_d of these lipoproteins.

The male-female differences in apoE distribution in VLDL and HDL were statistically significant at $P < 0.01$.

The plasma decay curve for radiolabeled apoE-3 in one of the study subjects is illustrated in **Fig. 3** and was representative of all plasma curves. The decay was triexponential with approximately 25% of the radiolabeled apoE remaining in plasma at 24 hr and 3% at day 7. Less than 10% of the plasma radioactivity was trichloroacetic acid-soluble at any time point and elimination of the trichloroacetic acid-soluble radioactivity from total plasma activity did not significantly alter the shape or the area under the decay curve.

The residence time (RT = 1/fractional catabolic rate) was determined from the area under the plasma decay curve, and the production rate of apoE was calculated (**Table 5**). The mean residence time for apoE-3 in these normal subjects was 0.73 days and the mean production rate was 3.40 mg/kg-day. In comparison to the female study subjects, the males had similar apoE concentrations and tended to have shorter residence times and higher production rates for apoE. For the total study population,

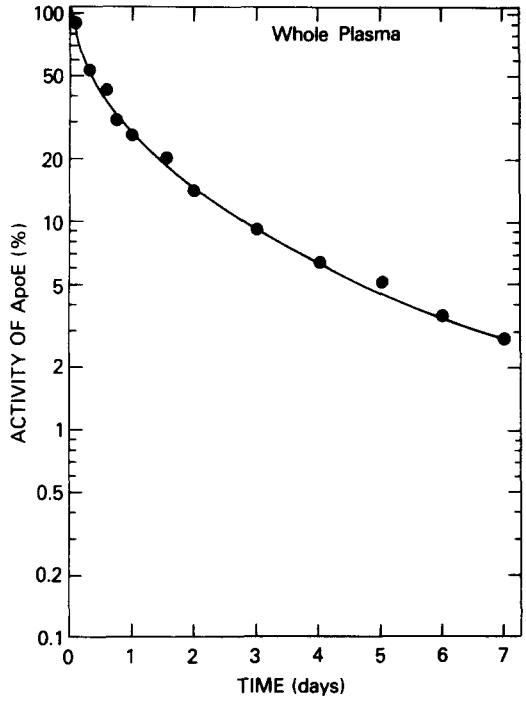

Fig. 3. The catabolism of apoE from plasma in a normal subject. Radiolabeled apoE-3 was reassociated with lipoproteins and injected into a normal subject. Plasma was isolated at various time intervals (see Methods) and the quantity of radioactivity was determined in 5 ml of plasma at each time point. The radioactivity was normalized to 1 at the first time point.

TABLE 5. Metabolic parameters of apoE-3 metabolism in normal subjects

Study Subject	Sex	Weight	Plasma Volume	ApoE	Residence Time	Production Rate
		kg	*ml*	*mg/dl*	*days*	*mg/kg-day*
A	F	57.1	2669	3.5	0.89	1.83
B-1	F	55.0	2521	4.5	0.83	2.49
B-2	F	57.7	3086	4.1	1.08	2.03
C-1	F	56.1	2303	5.9	0.60	4.04
C-2	F	56.4	2495	4.7	0.84	2.48
D	F	57.2	2479	4.5	0.71	2.75
E	M	90.8	3925	3.1	0.51	2.63
F-1	M	84.7	3722	6.3	0.71	3.90
F-2	M	84.3	4417	5.5	0.84	3.43
G	M	73.6	4102	6.6	0.49	7.51
H	M	75.0	4194	5.8	0.74	4.38
I	M	76.5	3971	3.2	0.50	3.32
Female (n = 6)		57.2 ± 1.0[a,b]	2592 ± 269[b]	4.5 ± 0.8[c]	0.83 ± 0.16[d]	2.60 ± 0.78[e]
Male (n = 6)		80.8 ± 6.8	4055 ± 240	5.1 ± 1.5	0.63 ± 0.15	4.20 ± 1.73
Total (n = 12)		68.7 ± 13.5	3324 ± 802	4.8 ± 1.2	0.73 ± 0.18	3.40 ± 1.52

[a] Mean ± SD.
Female-male comparison: [b], $P < 0.001$; [c], $P > 0.4$; [d], $P < 0.06$; [e], $P < 0.07$.

the plasma apoE concentrations correlated with the production rate ($r = 0.73$, $P < 0.01$) but did not correlate with the residence time ($r = -0.11$, $P > 0.7$).

The specific activity decay curve of apoE within each lipoprotein subfraction compared to the plasma curve in one subject is illustrated in **Fig. 4,** and is similar to the curves observed in all other subjects. The following conclusions were ascertained from the results of these subfraction studies. *1*) The decay of apoE-3 in the lipoprotein subfractions was most rapid within VLDL. *2*) There was a slight delay in the decay of radiolabeled apoE within IDL and LDL, i.e., the specific activity 30 min after injection was equal to or slightly higher than the specific activity 10 min after injection, and the initial slopes of the IDL and LDL decay curves were less than the initial slope of the plasma curve. This result suggests that there is at least a partial precursor-product relationship of apoE between VLDL and IDL/LDL. *3*) The apoE-3 decay in HDL was the slowest of all lipoprotein subfractions. *4*) The radiolabeled apoE in the d 1.21 g/ml infranate was removed rapidly and its decay curve was similar in shape to the apoE curve for VLDL.

DISCUSSION

ApoE has been intensively studied, and it has been proposed that apoE is important in regulating the metabolism of triglyceride-rich lipoproteins (10–14). Variants of apoE including apoE-1 and apoE-2 as well as apoE deficiency have been associated with elevations of plasma lipoproteins within VLDL and IDL and the type III phenotype (3, 4, 8, 9). The elevation of VLDL and IDL particles containing apoB-48 and apoB-100 in the plasma of patients with type III hyperlipoproteinemia indicates that apoE is important in the metabolism of lipoproteins synthesized by both the liver and intestine (25, 26).

Despite the interest in apoE, there is a paucity of studies evaluating the in vivo kinetics of apoE metabolism in humans; the only previous study being our initial study of apoE metabolism in normal and type III hyperlipoproteinemic subjects (27). We have, therefore, initiated a systematic study of the kinetics of the metabolism of the different allelic forms of apoE in humans.

In the present studies, the iodination of apoE was performed by a modification of the iodine monochloride method (16). We have previously shown that apoE maintains its antigenicity after iodine monochloride iodination but not with chloramine-T or lactoperoxidase iodination (15). Guanidine-HCl was used in the iodination procedure to eliminate aggregation of apoE and decrease its surface active properties. Utilizing this method all of the isoforms of apoE were iodinated with high efficiency.

Radiolabeled apoE was shown to have similar physicochemical properties to unlabeled apoE when analyzed by SDS-PAGE and isoelectrofocusing. The distribution of radiolabeled apoE was also similar to unlabeled apoE in plasma following in vitro incubation or at the 10 min point following injection into normal subjects. The distribution of apoE was determined following ultracentrifugation and by gel permeation chromatography. Using both techniques the specific activity of apoE was the same in all lipoprotein fractions. In addition, the

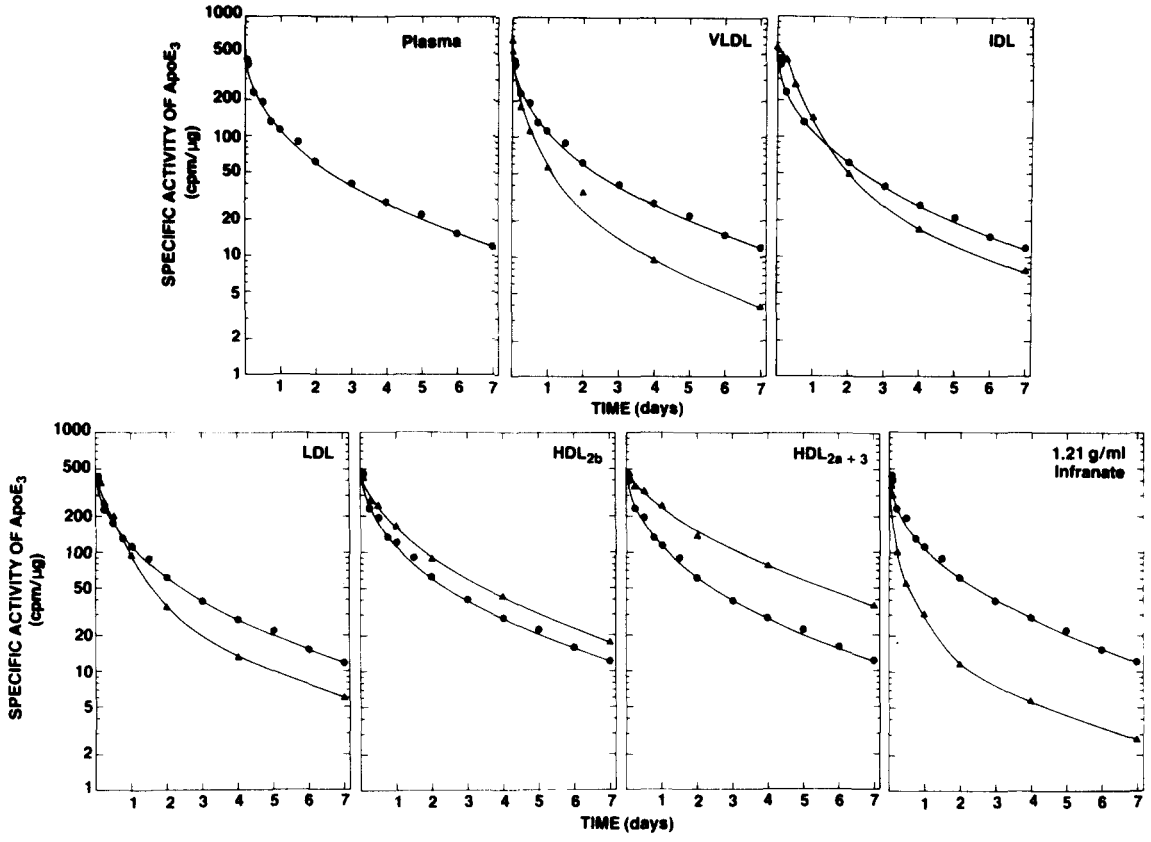

Fig. 4. The specific activity decay curves of apoE from plasma and lipoprotein subfractions. Following the injection of apoE reassociated with lipoproteins, 5 ml of plasma was obtained at various time intervals and fractionated by ultracentrifugation. The specific activity decay curves of apoE were determined for each lipoprotein subfraction. The plasma specific activity decay curve (● – – – ●) is plotted in each panel to facilitate the comparisons with the different lipoprotein subfractions (▲ – – – ▲).

decay of apoE radiolabeled as an apolipoprotein and reassociated with lipoproteins was identical to apoE radiolabeled on VLDL. These results, therefore, indicate that radiolabeled apoE is an effective tracer to study apoE metabolism.

ApoE metabolism was studied in healthy adult males and females. ApoE is catabolized very rapidly with a residence time of 0.73 days and a production rate of 3.40 mg/kg-day. This residence time is faster than that reported for apoA-I (28), apoA-II (28), apoA-IV (29), apoB-100 (30), apoC-II (31), or apoC-III (31). ApoB-48 is the only apolipoprotein with a shorter residence time (32, 33). It is of interest that apoE is catabolized more slowly than VLDL is converted to IDL and LDL utilizing apoB-100 as a marker (30), and more slowly than chylomicrons are catabolized from plasma (34).

The initial specific activities of apoE on plasma lipoproteins were equal; however, the rates of catabolism of apoE on these lipoproteins were different. The catabolism of apoE was the most rapid on VLDL, slowest on HDL, and intermediate on IDL and LDL. Because of the exchange of apoE between lipoprotein particles (35, 36), it is not possible to ascertain the lipoproteins on which apoE is either synthesized or catabolized. It is, however, clear that the rate of exchange is slow and may approximate the rate of apoE catabolism. If the exchange rate were rapid, the specific activity decay curves in each subfraction would be equal, which was not observed (Fig. 4). In addition, there is evidence for at least a partial precursor-product relationship between apoE on VLDL and the apoE associated with IDL-LDL.

The apoE in the d 1.21 g/ml infranate is of interest. The decay rate within the d 1.21 g/ml infranate is rapid; however, it is unclear as to the origin of the apoE in this fraction or its significance. If it was simply apoE that was dissociated by ultracentrifugation (37), one would expect it to parallel the plasma curve or the HDL curve which was not the case. It is possible that the d 1.21 g/ml infranate decay curve may reflect the catabolism of the proteolytic fragments of apoE (38). At this time it is unknown if the apoE in the d 1.21 g/ml infranatant reflects apoE dissociated preferentially from

VLDL, if it is a distinct metabolic pool of apoE in plasma, or if it represents an artifact of the isolation procedures.

The metabolism of apoE may be different in males and females. When compared to females, the residence time of apoE in males tends to be shorter, and the production rate is increased resulting in similar plasma concentrations of apoE. In previous studies, females have been reported to have higher plasma apoE concentrations (2) and this may be due to the increased apoE residence time. The cause of the increased residence time in females is unclear; however, it may reflect the increased percentage of apoE associated with the slower catabolized HDL apoE pool in females.

The combined results from the kinetic studies of apoE metabolism are consistent with the concept that apoE is synthesized by the liver (39) and secreted on VLDL (40) and HDL (41, 42). The apoE in VLDL may be either catabolized on triglyceride-rich lipoproteins or slowly exchanged between VLDL and HDL. The apoE in HDL may serve as a reservoir for the transfer of apoE to newly secreted triglyceride-rich lipoproteins, where it functions to modulate the hepatic uptake of remnants of triglyceride-rich particles. The plasma metabolism of apoE is complex, with apoE exchanging between lipoprotein particles and possibly being catabolized on more than one type of lipoprotein particle. With the methods developed in this study it will now be possible to study the metabolism of apoE-2 and apoE-4 in normal individuals and subjects with hyperlipoproteinemia in order to gain further insights into the functions of apoE and the abnormalities of apoE metabolism in patients with dyslipoproteinemias.

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