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Research Article

Apolipoprotein E (apoE) is important in modulating the catabolism of remnants of triglyceride-rich lipoprotein particles. It is a polymorphic protein with the three common alleles coding for apoE2, apoE3, and apoE4. ApoE3 is considered the normal isoform, while apoE4 is associated both with hypercholesterolemia and type V hyperlipoproteinemia. We quantitated the kinetics of metabolism of apoE4 in 19 normolipidemic apoE3 homozygotes and 1 normolipidemic apoE4 homozygote, and compared this with the metabolism of apoE3 in 12 normolipidemic apoE3 homozygotes. In the apoE3 homozygous subjects, apoE4 was catabolized twice as fast as apoE3, with a mean plasma residence time of 0.37 +/- 0.01 d (+/- SEM) and 0.73 +/- 0.05 (P less than 0.001), respectively. When plasma was fractionated into the lipoprotein subclasses, the greatest amount of labeled apoE4 was present on very low density lipoproteins, while the largest fraction of labeled apoE3 was associated with high density lipoproteins. The plasma apoE concentration was decreased in an apoE4 homozygote compared with the apoE3 homozygotes (3.11 mg/dl vs. 4.83 +/- 0.35 mg/dl). The reduced apoE4 concentration was entirely due to a decreased apoE4 residence time in the apoE4 homozygote (0.36 d vs. 0.73 +/- 0.05 d for apoE3 in apoE3 homozygotes). These results indicate that apoE4 is kinetically different than apoE3, and suggest that the presence of apoE4 in hypercholesterolemic and type [...]

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Abnormal In Vivo Metabolism of Apolipoprotein E₄ in Humans

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Abstract

Apolipoprotein E (apoE) is important in modulating the catabolism of remnants of triglyceride-rich lipoprotein particles. It is a polymorphic protein with the three common alleles coding for apoE₂, apoE₃, and apoE₄. ApoE₃ is considered the normal isoform, while apoE₄ is associated both with hypercholesterolemia and type V hyperlipoproteinemia. We quantitated the kinetics of metabolism of apoE₄ in 19 normolipidemic apoE₃ homozygotes and 1 normolipidemic apoE₄ homozygote, and compared this with the metabolism of apoE₃ in 12 normolipidemic apoE₃ homozygotes. In the apoE₃ homozygous subjects, apoE₄ was catabolized twice as fast as apoE₃, with a mean plasma residence time of 0.37 ± 0.01 d (\pm SEM) and 0.73 ± 0.05 ($P < 0.001$), respectively. When plasma was fractionated into the lipoprotein subclasses, the greatest amount of labeled apoE₄ was present on very low density lipoproteins, while the largest fraction of labeled apoE₃ was associated with high density lipoproteins.

The plasma apoE concentration was decreased in an apoE₄ homozygote compared with the apoE₃ homozygotes (3.11 mg/dl vs. 4.83 ± 0.35 mg/dl). The reduced apoE₄ concentration was entirely due to a decreased apoE₄ residence time in the apoE₄ homozygote (0.36 d vs. 0.73 ± 0.05 d for apoE₃ in apoE₃ homozygotes).

These results indicate that apoE₄ is kinetically different than apoE₃, and suggest that the presence of apoE₄ in hypercholesterolemic and type V hyperlipoproteinemic individuals may play an important pathophysiological role in the development of these dyslipoproteinemias.

Introduction

Human apolipoprotein E (apoE)¹ is a polymorphic plasma protein with three common isoforms present in the normal population (1, 2). These three isoforms, apoE₂, apoE₃, and apoE₄, are coded by three separate alleles, E², E³, and E⁴, that are inherited in a codominant fashion at a single genetic locus (2). Type III hyperlipoproteinemia is associated with homozygosity for the E² allele (2, 3) while the E⁴ allele is associated with type V hyperlipoproteinemia (4) and increased plasma cholesterol levels (5–7).

Preliminary results of these investigations were presented at the American Heart Association Meeting, Dallas, TX, November, 1982.

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1. Abbreviations used in this paper: apo, apolipoprotein; IDL, intermediate density lipoprotein.

The primary amino acid sequence of the apolipoproteins for the three common isoforms of apoE are known. These isoforms differ only at amino acid positions 112 and 158, with apoE₂ having cysteines at both positions, apoE₃ having a cysteine at position 112 and an arginine at position 158, and apoE₄ containing arginines at both positions (8). ApoE₂ has been previously shown to be catabolized more slowly than apoE₃ in in vivo human studies (9) and to have reduced receptor binding properties in in vitro cell membrane binding studies when compared with apoE₃ (10, 11). Therefore, apoE₂ is both functionally and metabolically different from apoE₃, and the homozygous apoE₂ phenotype is linked both genetically and metabolically with type III hyperlipoproteinemia (12).

The ability of apoE₄ to bind to cellular membrane receptors has also been reported. ApoE₄ had the same binding properties as apoE₃ (10), and we interpreted these results as indicating that apoE₄ is a metabolically normal isoform. However, the findings that apoE₄ is associated with type V hyperlipoproteinemia and with increased plasma cholesterol levels is consistent with apoE₄ being an abnormal isoform (4–7). In order to gain further insight into the metabolism of apoE₄, we have compared the in vivo kinetics of radiolabeled apoE₃ and apoE₄ metabolism in normal individuals with a homozygous apoE₃ phenotype as well as in a subject with a homozygous apoE₄ phenotype.

Methods

Study subjects. 19 normal subjects were studied with a homozygous apoE₃ phenotype; there were 9 males and 10 females between the ages of 18 and 24 yr. In addition, a 22-yr-old normal male with a homozygous apoE₄ phenotype was studied. All of these individuals were hospitalized for study in the Clinical Center of the National Institutes of Health. 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 illnesses. All subjects gave informed consent and the study protocol was approved by the Institute Review Board of the National Heart, Lung, and Blood Institute.

Isolation of apoE. ApoE₃ and apoE₄ were isolated from an individual homozygous for the E³ allele and E⁴ allele, respectively. ApoE was isolated and characterized as previously described (13). Briefly, very low density lipoprotein (VLDL) was obtained by ultracentrifugation ($d < 1.006$ g/ml) and delipidated with chloroform/methanol 2:1 (vol/vol). ApoE was separated from the other apolipoproteins by heparin affinity and Sephacryl S-200 gel permeation chromatography. Purified apoE migrated as a single electrophoretic band on sodium dodecyl sulfate and pH 8.4 urea polyacrylamide gel electrophoresis.

Iodination of apoE. ApoE was iodinated as previously described by a modification of the iodine monochloride method (14). Lyophilized apoE was redissolved in a buffer of 6 M guanidine-HCl and 1 M glycine (pH 8.5), 5 mCi of Na¹²⁵I or Na¹³¹I was added to the solution and followed by the slow addition of iodine monochloride during vortexing of the sample. ApoE was iodinated with an efficiency of 30–50% with incorporation of ~0.5 mol of iodine per mole of apoE. Radiolabeled apoE was incubated with plasma for 30 min at 37°C, the density of the plasma adjusted to 1.21 g/ml with solid KBr, and centrifuged for 2.2×10^8 g/min. The $d < 1.21$ g/ml supernatant was isolated by tube slicing and dialyzed against 150 mM NaCl, 100 mM Tris-HCl (pH 7.4), and 0.01%

EDTA. Human serum albumin was added at a final concentration of 1% and the solution sterilized by filtration through a 0.22- μ m filter. Each preparation was tested for pyrogens before injection into the study subjects.

Study protocol. The study subjects were placed on a defined isoweight diet containing 42% carbohydrate, 42% fat, 16% protein, 200 mg of cholesterol per 1,000 kcal, and a polyunsaturated/saturated fat ratio of 0.1:0.3 for 10 d before beginning the apoE metabolic studies. The diet was changed to a liquid formula of the same nutrient composition given every 6 h beginning 3 d before the apoE injection, and continued for the duration of the study. This dietary regimen was required in order to maintain a metabolic steady state in which to study the kinetics of the metabolism of apoE. 3 d before the injection, the subjects were also started on potassium iodide (1,200 mg/d) and ferrous gluconate (900 mg/d) in divided doses. Subjects were injected intravenously with up to 25 μ Ci of 131 I-radiolabeled and 100 μ Ci of 125 I-radiolabeled apoE ~5.5 h after the previous meal. In some studies, the subjects received only one form of radiolabeled apoE while in others they received both forms. In addition, there were no alterations in the kinetics of metabolism of apoE if it was labeled with 125 I or 131 I, i.e., there was no isotope effect on the metabolism of apoE. Blood samples were obtained at 10 min and at 6, 12, 18, 24, 36, and 48 h, and then daily through day 7. All blood samples except the first one were drawn just before a meal (i.e., after a 6-h fast, while the first one was drawn after a 5.5-h fast).

Blood samples (20 ml) were collected into tubes containing EDTA at a final concentration of 0.1%. Blood was kept at 4°C and plasma separated by low speed centrifugation (2,000 rpm, 30 min) in a refrigerated centrifuge (4°C). Sodium azide and aprotinin (Boehringer Mannheim, Mannheim, Federal Republic of Germany) were added to each plasma sample at a final concentration of 0.05% and 200 Kallikrein inhibitor units/ml, respectively. 1 ml of plasma was frozen at -20°C for apoE quantitation. The plasma lipoproteins were isolated by ultracentrifugation (15), the radioactivity quantitated, and the apoE concentration determined. The radioactivities in plasma and the lipoprotein subfractions were quantitated in a gamma counter (Autogamma 5260; Packard Instrument Co., Inc., Downers Grove, IL).

Analytical methods. In each subject the apoE phenotypes were ascertained by analytical isoelectrofocusing (4). Plasma cholesterol and triglycerides were quantitated on an enzymic analyzer (Gilford System 3500; Gilford Instrument Laboratories, Inc., Oberlin, OH). High density lipoprotein (HDL) cholesterol was determined in plasma after dextran sulfate precipitation (16). All other lipid and lipoprotein analyses were performed by the methods of the Lipid Research Clinics (17). ApoE was quantitated by a double antibody radioimmunoassay (13). The residence

time (1/fractional catabolic rate) was determined from the area under the plasma decay curve by a multiexponential computer curve-fitting technique (18). The production rate was calculated from the following formula: production rate = (apoE concentration \times plasma volume)/(residence time \times 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 of the radiolabeled apoE. Intergroup comparisons were performed using Student's *t* test.

Results

The clinical characteristics of the study subjects are given in Table I. These data include the individuals who had radioiodinated apoE₃ studies that we have previously reported (14).

Lipoprotein distribution of radiolabeled apoE₃ and apoE₄. ApoE is present on all classes of lipoprotein particles but is found predominantly on VLDL and HDL. Radiolabeled apoE₃ and apoE₄ were injected into normal subjects with a homozygous apoE₃ phenotype and a plasma sample was obtained from each individual at 10 min. The distribution of radiolabeled apoE in the lipoprotein subfractions was determined after isolation of the lipoproteins by ultracentrifugation. As presented in Table II, HDL contained the largest amount of apoE₃, followed by VLDL and the *d* > 1.21 g/ml infranate. The distribution of radiolabeled apoE₄ differed markedly in these normal subjects. ApoE₄ was present primarily on VLDL, with much less present on HDL when compared with apoE₃ (Table II). There was approximately the same quantity of apoE₃ and apoE₄ on the other lipoprotein subfractions. Therefore, apoE₃ and apoE₄ differ markedly in their affinity for different classes of lipoprotein particles.

Radiolabeled apoE kinetic studies in subjects with a homozygous apoE₃ phenotype. Radioiodinated apoE₃ and/or apoE₄ were injected into normal subjects with an apoE₃ phenotype and plasma samples obtained periodically over a 7-d period. The radioactivity was determined at each time point in plasma and the isolated lipoprotein subfractions. The plasma cholesterol, triglyceride, and apoE concentration did not differ between the groups of normal subjects receiving either apoE₃ or apoE₄ (Table

Table I. Clinical and Laboratory Data for Subjects Undergoing Metabolic Study

ApoE phenotype	Male	Female	Age yr	Cholesterol	Triglyceride	Cholesterol			Plasma apoE‡	
						VLDL mg/dl	LDL mg/dl	HDL mg/dl		
Subjects for apoE ₃ kinetics studies (n = 12)	E ₃	6	6	20-23*	125-185	54-88	5-13	64-112	28-94	3.1-6.6
Subjects for apoE ₄ kinetic studies (n = 19)	E ₃	4	15	19-23	130-210	60-130	8-20	65-120	35-70	3.7-7.1
Subject with homozygous apoE ₄ phenotype (n = 1)	E ₄	1	—	22	175	75	15	93	67	3.1

* Range of the subjects. ‡ Normal range, 5.7 \pm 1.4 mg/dl (\pm SD, reference 13).

Table II. Lipoprotein Distribution of Radiolabeled ApoE in Normolipidemic ApoE₃ Homozygotes

	ApoE ₃ *	ApoE ₄ ‡
	%	%
VLDL	23.1±1.7§	39.8±3.9
IDL	4.5±0.3	5.2±0.4
LDL	11.9±0.7	12.3±0.7
HDL	41.4±1.9	26.3±2.3
<i>d</i> < 1.21 g/ml	20.9±1.0	16.6±1.5

* *n* = 12.

‡ *n* = 19.

§ ±SEM.

^{||} *P* < 0.01.

III). Fig. 1 illustrates representative catabolic decay curves for apoE₃ and apoE₄ injected simultaneously into a normal subject. ApoE₄ is catabolized much more rapidly than apoE₃. Both decay curves are multiexponential, indicating multiple metabolic compartments for apoE. The residence time of apoE was determined in each individual, using computer-assisted multiexponential curve fitting (18) in order to quantitate the catabolic difference between apoE₃ and apoE₄. The residence time of apoE₄ was ~50% of the residence time of apoE₃ (i.e., there was a much greater fractional catabolic rate for apoE₄, Table III).

In order to insure that the differences in the metabolism between apoE₃ and apoE₄ were not due to an artifact during one isolation procedure or unique to the apoE₄ from one particular subject, these kinetic investigations utilized apoE₄ from a total of four different apoE isolations from two different subjects, and apoE₃ from a total of three different apoE isolations from two different subjects. There were no significant differences in the catabolic rates for the different lots of apoE₃ or the different lots of apoE₄. In addition, in one subject the catabolic rates of apoE₄ isolated from two different subjects was directly compared; the catabolic decay curves are illustrated in Fig. 2. It is apparent that the decay curves are virtually identical. We have therefore concluded that the differences in the catabolism between apoE₃ and apoE₄ are not due to an artifact in the isolation procedure or to a unique form of apoE₄ from a particular subject, but result from the known primary amino acid sequence difference between apoE₃ and apoE₄.

The catabolic decay curves for apoE₄ in the individual li-

Table III. Kinetics of ApoE Metabolism in Normolipidemic ApoE₃ Homozygotes

	ApoE ₃ *	ApoE ₄ ‡
Cholesterol (mg/dl)	169±8§	168±6
Triglyceride (mg/dl)	124±12	134±14
ApoE (mg/dl)	4.83±0.35	4.73±0.38
Residence time (d)	0.73±0.05	0.37±0.01

* *n* = 12.

‡ *n* = 19.

§ Mean±SEM of the values obtained after 6-h fasts during the kinetic study.

^{||} *P* < 0.001.

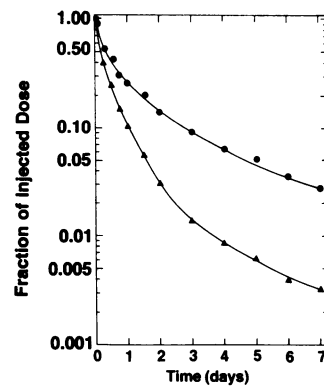


Figure 1. Catabolism of apoE₃ and apoE₄ in a normolipidemic subject homozygous for apoE₃. ApoE₃ (—●—) and apoE₄ (—▲—) were radioiodinated with ¹²⁵I and ¹³¹I, respectively, reassociated with lipoproteins, and injected into the subjects at time 0. The amount of tracer remaining in the plasma was quantitated over a 7-d time period and normalized to 1 at time 0.

poprotein subfractions are illustrated in Fig. 3. In each panel the corresponding decay curve for apoE₃ is given for comparison. ApoE₄ is catabolized most rapidly from VLDL and at the slowest rate from HDL. In addition, these results for apoE₄ are similar to our previously reported data for apoE₃ in normal subjects where the catabolic rates from intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and HDL_{2b} were similar to the catabolic rate for whole plasma (14). ApoE₄ is catabolized more rapidly than apoE₃ from the plasma and all of the lipoprotein subfractions (e.g. VLDL, IDL, LDL, and HDL), however it is catabolized at nearly the same rate as apoE₃ in the *d* > 1.21 g/ml infranate. Thus, apoE₄ is catabolized more rapidly than apoE₃ in plasma and the lipoprotein subfractions. Even though apoE does not normally exist as a free apolipoprotein in plasma, the results from the *d* > 1.21 g/ml infranate would suggest that the apoE in this lipoprotein-free fraction may be derived from a separate intravascular metabolic pool and does not reflect the catabolic behavior of apoE from any particular class of lipoprotein particles. This is because the *d* > 1.21 g/ml infranate decay curve is different than any of the lipoprotein subfraction decay curves and because both apoE₃ and apoE₄ are catabolized at the same rate from it.

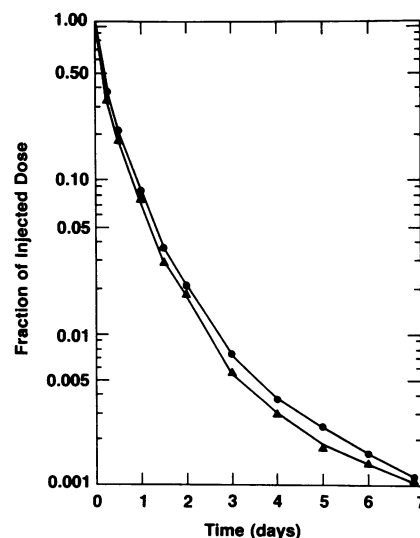


Figure 2. Catabolism of apoE₄ isolated from two different subjects homozygous for apoE₄. The subject into whom radioiodinated apoE₄ was injected was homozygous for apoE₃ and the study was performed as outlined for Fig. 1.

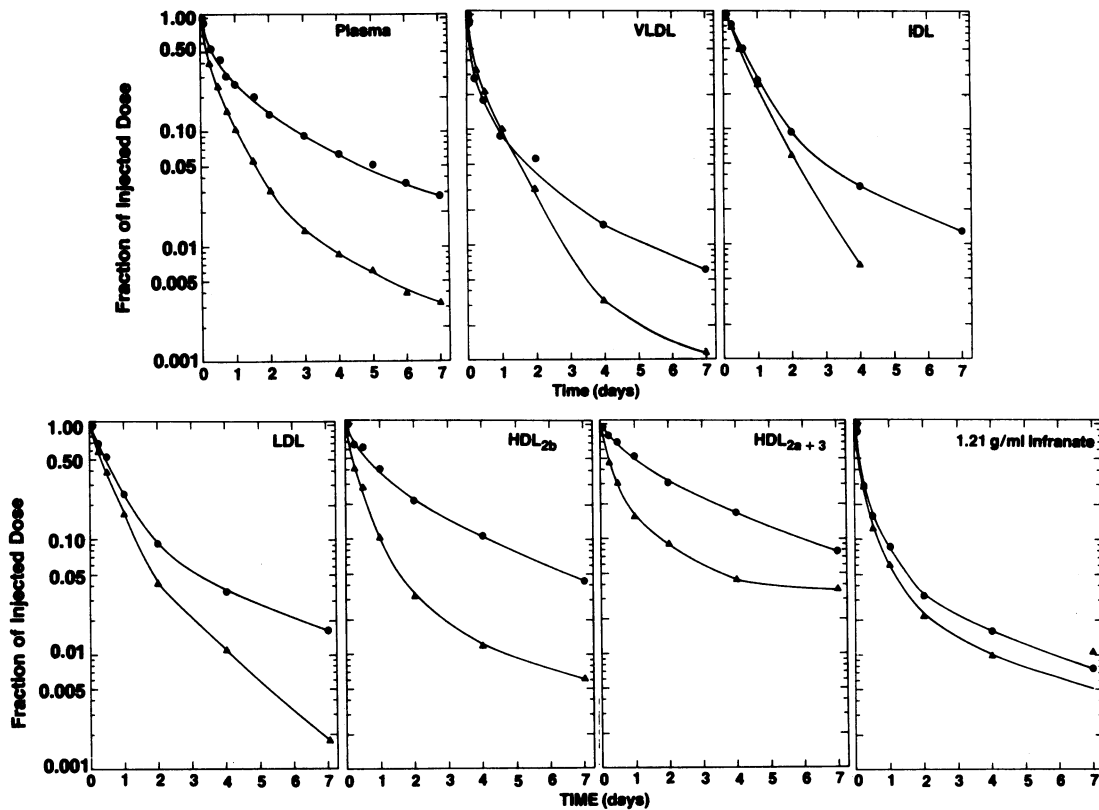


Figure 3. Catabolism of apoE₃ and apoE₄ in the lipoprotein subfractions of a normolipidemic subject homozygous for apoE₃. ApoE₃ iodinated with ¹²⁵I (—●—) and ¹³¹I apoE₄ (—▲—) were injected into a subject, plasma was obtained, and lipoprotein fractions were sepa-

rated at the indicated times. The decrease of radiolabeled apolipoproteins from each lipoprotein fraction is plotted over time after normalization to 1 at time 0.

Radiolabeled apoE₄ kinetic studies in a patient with a homozygous apoE₄ phenotype. The clinical characteristics of an individual with a homozygous apoE₄ phenotype are given in Table I. This subject was a normal healthy volunteer without a history of any significant medical problems, and his apoE phenotype was established while screening normolipidemic volunteer subjects. His plasma lipid and lipoprotein concentrations are normal while his cholesterol concentration was in the low normal range (Table I). The kinetics of the metabolism of ¹²⁵I-apoE₃ and ¹³¹I-apoE₄ were determined in this individual, and the plasma and lipoprotein subfraction catabolic decay curves for apoE₃ and apoE₄ are illustrated in Fig. 4. The decay for both types of apoE from plasma are multiexponential with the decay of apoE₄ being much faster than apoE₃. As was determined in the homozygous apoE₃ subjects, apoE₄ was catabolized more rapidly than apoE₃ in VLDL, IDL, LDL, and HDL. The catabolism of apoE₃ and apoE₄ were similar in the *d* > 1.21 g/ml infranate.

The kinetic parameters of apoE metabolism in this apoE₄ subject are presented in Table IV. The plasma apoE concentration in this individual was ~65% of the apoE concentration in the other subjects with a homozygous apoE₃ phenotype. The residence times for apoE₃ and apoE₄ were similar to the corresponding residence times in the apoE₃ individuals, with the apoE₄ residence time being approximately half the residence time of apoE₃. The plasma production rate for apoE₄ was increased when compared with the production rate of apoE₃ in apoE₃ individuals. Therefore, the apoE isoform was more important than the phe-

notype of the individual in determining the catabolic rate for apoE in this subject. The decreased apoE concentration in the apoE₄ subject resulted from rapid catabolism of apoE and not from a decreased production rate, in that there was actually an increased production rate of apoE.

Discussion

Current data indicate that apoE is important in modulating the catabolism of remnants of triglyceride-rich lipoprotein particles. ApoE₂, which is associated with type III hyperlipoproteinemia, has been shown to be abnormal in both in vivo and in vitro studies (1-3, 9-12). ApoE₄ is associated with hypercholesterolemia (5-7) and with type V hyperlipoproteinemia (4), but has been reported to be normal in in vitro receptor binding studies (10). In order to gain further insights into the function of apoE₄ in lipoprotein metabolism, we investigated the in vivo metabolism of radioiodinated apoE₄ in normal humans. We have previously described in detail and validated the methods used for these kinetic studies (14). The studies validating these methods have demonstrated that apoE iodinated on a lipoprotein particle is metabolized at the same rate as isolated iodinated apoE, and that iodinated apoE reassociated with lipoproteins in plasma either in vivo or in vitro has a constant specific activity in the different lipoprotein subfractions (14).

ApoE₄ was found to differ from apoE₃ in its distribution on lipoprotein particles. A higher percentage of apoE₄ was bound to VLDL with less on HDL when compared with apoE₃. The

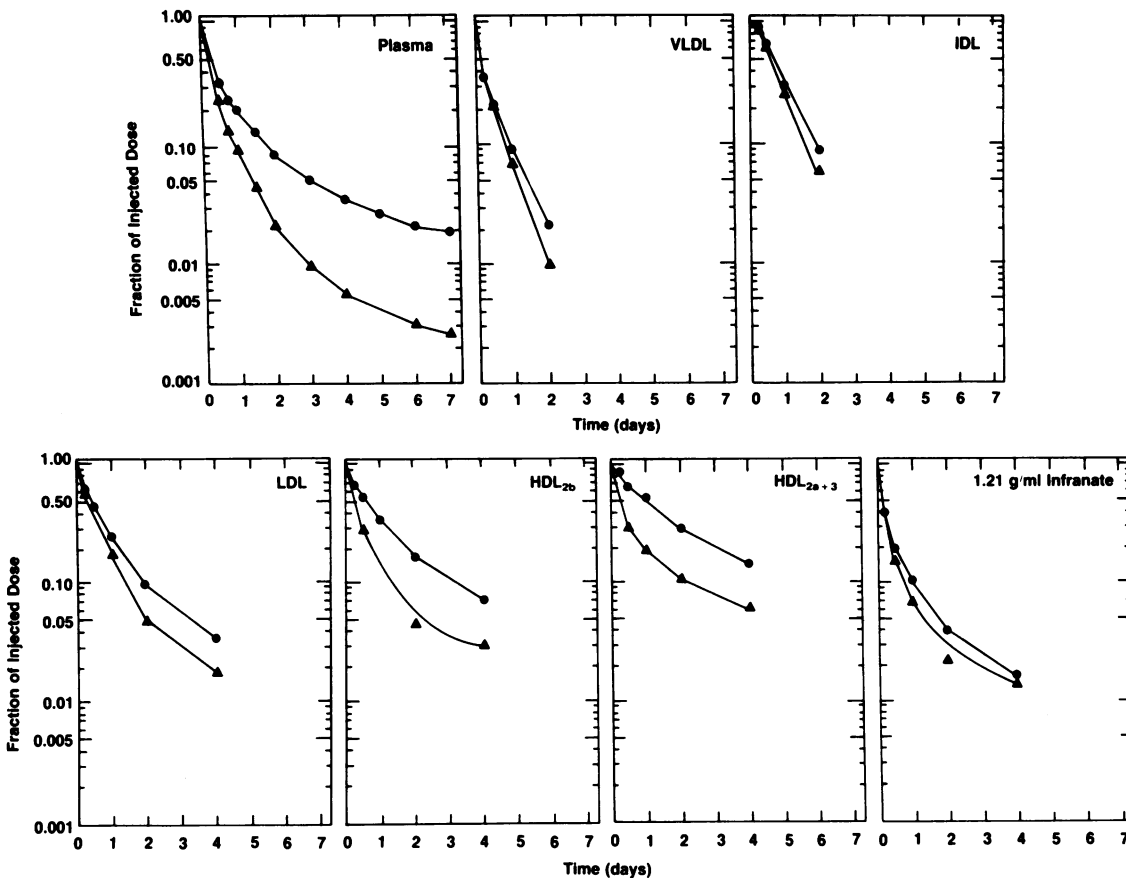


Figure 4. Catabolism of apoE₃ and apoE₄ from plasma and lipoprotein subfractions in a normolipidemic subject homozygous for apoE₄. ¹²⁵I apoE₃ (—●—) and ¹²⁵I apoE₄ (—▲—) were injected into a normolipidemic apoE₄ homozygote, and the decrease of labeled apolipoproteins from plasma was determined.

reason for this difference in distribution is as yet unknown. One possibility is that the additional positive charge on apoE₄ may lead to a higher affinity to VLDL through protein-protein or protein-lipid interactions. A second possibility is that apoE₄, with no cysteines, can't form apoE/apoA-II (19) or other mixed dimers that may lead to an altered lipoprotein particle distribution of the E apolipoprotein. It is clear, however, that apoE₄ has an altered lipoprotein particle distribution when compared with apoE₃.

In addition to altered lipoprotein particle distribution, apoE₄ was kinetically abnormal in individuals with a homozygous

apoE₃ phenotype. ApoE₄ was catabolized with a plasma residence time of approximately half the plasma residence time of apoE₃. The apoE₄ was also catabolized more rapidly than apoE₃ from all lipoprotein subfractions, except for the *d* > 1.21 g/ml infranate. The rapid metabolism of apoE₄ may be due to at least four different reasons. First, since a higher percentage of apoE₄ is present on VLDL and if the apoE₄ is catabolized with VLDL through a receptor-mediated endocytotic process, it would be anticipated to be catabolized more rapidly due to its association with VLDL. Second, if apoE/apoA-II and other apoE dimers are metabolized at a slower rate than apoE monomers, apoE₄ would be metabolized more rapidly than apoE₃ because it is devoid of cysteines. Third, apoE₄ may interact in vivo with the apoE receptor with a higher affinity than apoE₃, which could lead to a faster catabolism of apoE₄ and remnant particles. Fourth, apoE₄ may facilitate the process of conversion of VLDL remnants into LDL more readily than apoE₃. This conversion process could occur via binding of the remnant particles to hepatic receptors (either the LDL (B/E) receptor and/or the apoE receptor) with a portion of the internalized remnant particles being resecreted as LDL via a retroendocytotic process (20), or the conversion could be extracellular and mediated by lipolytic enzymes, including hepatic lipase. Regardless of the mechanism, the ultimate conversion of VLDL remnants to LDL may be facilitated more by apoE₄ than by apoE₃.

The plasma apoE concentration in the individual with the homozygous apoE₄ phenotype was decreased compared with apoE₃ subjects, and was also decreased in three other normoli-

Table IV. Kinetics of ApoE Metabolism*

	E ₃ Phenotype‡	E ₄ Phenotype
Chol. (mg/dl)	169±8	180
TG (mg/dl)	124±12	190
ApoE (mg/dl)	4.83±0.35	3.11
Residence time (d)	0.73±0.05	0.36
Production rate (mg/kg per d)	2.84±0.31	4.88

* Kinetics of apoE₃ metabolism in apoE₃ homozygotes and apoE₄ metabolism in the apoE₄ homozygote.

‡ *n* = 12.

§ *n* = 1.

^{||} Mean±SEM of the values obtained after 6-h fasts during the kinetic study.

pidemic apoE₄ individuals in which we have quantitated apoE levels. The metabolic reason for the low plasma level of apoE₄ was a rapid fractional catabolic rate, with the apoE₄ production rate being actually increased in the apoE₄ subject when compared with the apoE₃ subjects. Therefore, the low plasma concentration of apoE₄ was due to rapid catabolism. In the apoE₄ subject, the rate of catabolism of apoE₄ was faster than that of apoE₃, and the catabolic rates were very similar to the apoE₃ and apoE₄ catabolic rates in the apoE₃ subjects. Therefore, the catabolic rate of apoE in this normolipidemic subject was much more dependent on the isoform of the apoE than on the apoE phenotype of the individual and is consistent with the mechanism for apoE catabolism being similar in normolipidemic apoE₃- and apoE₄-homozygous individuals. The normal lipid and lipoprotein concentrations of the apoE₄ subject indicates that the apoE concentration was sufficient for normal lipoprotein metabolism in this individual.

These results, combined with the previous epidemiologic studies, are interpreted as indicating that apoE₄ is an abnormal apoE isoprotein. This evidence includes: (a) an association of the E⁴ allele with type V hyperlipoproteinemia (4), (b) an association of the E⁴ allele with increased plasma and LDL cholesterol concentrations (5–7), (c) an altered lipoprotein distribution of apoE₄, (d) a decreased plasma apoE concentration in subjects with a homozygous apoE₄ phenotype, and (e) rapid catabolism of apoE₄ compared with apoE₃ in both apoE₃ and apoE₄ subjects, establishing that apoE₄ is metabolically different than apoE₃.

We propose the following conceptual scheme, based on these results, to explain the observed effects of the different forms of apoE on the metabolism of remnant particles and LDL. Within this framework it is assumed that apoE has both an effect on the conversion of VLDL remnants into LDL and on the direct catabolism of remnants of triglyceride-rich lipoprotein particles by hepatic receptors. In addition, it is assumed that normal metabolism is metabolism in normolipidemic subjects with the homozygous apoE₃ phenotype.

In subjects with a homozygous apoE₂ phenotype, the apoE is defective in both its ability to bind to the hepatic apoE receptor (10) and in the conversion of remnants to LDL (21–23). This results in an accumulation of remnants of triglyceride-rich lipoprotein particles and a decreased uptake of cholesterol-containing lipoproteins by the liver, due to a block in remnant uptake and a decrease in the conversion to LDL. The liver compensates by up-regulating the LDL receptor pathway, which further decreases plasma LDL concentrations (24). These combined effects result in a decreased catabolic rate for apoE₂, an increase in plasma remnant concentration, a decreased LDL concentration, an up-regulated LDL receptor pathway, and, presumably, an increased cholesterol synthesis pathway. If the individual also has an additional cause for hyperlipidemia, type III hyperlipoproteinemia develops, along with an increased risk for premature cardiovascular disease (3).

The evidence is less complete for the effect of apoE on lipoprotein metabolism in individuals with a homozygous apoE₄ phenotype; however, the present evidence is consistent with apoE acting at the same sites as in apoE₂ subjects, but with an effect opposite that of apoE₂. We propose that apoE₄ is more effective than apoE₃ in modulating direct remnant uptake and in converting remnants to LDL. This change in apoE metabolism results in rapid catabolism of apoE₄, rapid catabolism of cholesterol-enriched remnant particles, a down-regulation of the LDL

receptor pathway, increased conversion of VLDL remnants to LDL, and an increased LDL concentration due to both increased conversion from IDL and decreased LDL catabolism.

The mechanism for the association of apoE₄ with type V hyperlipoproteinemia is unknown; however, apoE may modulate the hydrolysis of triglycerides on large triglyceride-rich lipoprotein particles (25, 26). Alternatively, since it is on chromosome 19 (27), it may act as a marker for some other abnormal gene on chromosome 19, particularly since the apoE gene is on the same chromosome as the genes for apoC-I (28), apoC-II (29), and the LDL receptor gene (30).

In conclusion, the combined epidemiological and metabolic studies provide convincing evidence that the apoE₄ is a metabolically abnormal apoE isoform that is associated with significant changes in lipoprotein metabolism.

Acknowledgments

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