Abnormal in vivo metabolism of apoB-containing lipoproteins in human apoE deficiency

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Abstract **The present study was undertaken to elucidate the metabolic basis for the increased remnants and lipoprotein(a) [Lp(a)] and decreased LDL apolipoprotein B (apoB) levels in human apoE deficiency. A primed constant infusion of $^{13}C_6$-phenylalanine was administered to a homozygous apoE-deficient subject. apoB-100 and apoB-48 were isolated, and tracer enrichments were determined by gas chromatography-mass spectrometry, then kinetic parameters were calculated by multicompartmental modeling. In the apoE-deficient subject, fractional catabolic rates (FCRs) of apoB-100 in VLDL and intermediate density lipoprotein and apoB-48 in VLDL were 3×, 12×, and 12× slower than those of controls. On the other hand, the LDL apoB-100 FCR was increased by 2.6×. The production rate of VLDL apoB-100 was decreased by 45%. In the Lp(a) kinetic study, two types of Lp(a) were isolated from plasma with apoE deficiency: buoyant and normal Lp(a). ^{125}I-buoyant Lp(a) was catabolized at a slower rate in the patient. However, ^{125}I-buoyant Lp(a) was catabolized at twice as fast as ^{131}I-normal Lp(a) in the control subjects. In summary, apoE deficiency results in: *1)* a markedly impaired catabolism of VLDL/chylomicron and their remnants due to lack of direct removal and impaired lipolysis; *2)* an increased rate of catabolism of LDL apoB-100, likely due to upregulation of LDL receptor activity; *3)* reduced VLDL apoB production; and *4)* a delayed catabolism of a portion of Lp(a).**—Ikewaki, K., W. Cain, F. Thomas, R. Shamburek, L. A. Zech, D. Usher, H. B. Brewer, Jr., and D. J. Rader. **Abnormal in vivo metabolism of apoB-containing lipoproteins in human apoE deficiency. *J. Lipid Res.* 2004.** 45: **1302–1311.**

Supplementary key words apolipoprotein B • apolipoprotein E • kinetics • lipoprotein(a) • stable isotopes

Apolipoprotein E (apoE) functions as the ligand for receptors, including LDL receptor-related protein (LRP), VLDL receptor, and LDL receptor (1, 2), and is thus important for the metabolism of apoB-containing lipoproteins. apoE is also considered to be important for the lipolysis of triglyceride (TG)-rich lipoproteins (3–6) and has been reported to facilitate VLDL production in animal models (7, 8). apoE knockout mice have elevated VLDL and remnants and developed atherosclerosis on a chow diet (9). There have been several reported cases of humans with genetic apoE deficiency (10). All cases have type III hyperlipoproteinemia and some of the patients developed premature atherosclerosis in favor of the concept that apoE is crucial in human lipoprotein metabolism.

Two kinetic studies in apoE-deficient subjects have been reported. Schaefer et al. (10) performed a radiotracer study and reported an impaired VLDL metabolism, in particular apoB-48, in a single apoE-deficient patient. However, multicompartmental modeling was not employed, thus only VLDL apoB-100 and apo-B48 were mathematically analyzed. Gabelli et al. (11) reported, in an LDL kinetic study using the same patient, that the rate of catabolism of LDL apoB was increased in the apoE-deficiency patient despite the relatively lower affinity of the LDL particles for the LDL receptor. However, an overall scheme of apoB-containing lipoprotein metabolism in humans with apoE deficiency has not been established. To establish the effect of apoE deficiency on apoB-containing lipoproteins, we performed an in vivo kinetic study using stable, isotopically-labeled phenylalanine as a tracer in a patient with apoE deficiency.

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein related to the LDL structure, but differs from LDL in that

Manuscript received 21 January 2004 and in revised form 19 April 2004.

Published, JLR Papers in Press, April 21, 2004.
DOI 10.1194/jlr.M400020-JLR200

Abbreviations: apoE, apolipoprotein E; BMI, body mass index; FCR, fractional catabolic rate; GC-MS, gas chromatography-mass spectrometry; HDL-C, HDL cholesterol; IDL, intermediate density lipoprotein; Lp(a), lipoprotein(a); LRP, LDL receptor-related protein; PR, production rate; RT, residence time; TC, total cholesterol; TG, triglyceride; T/T, tracer/tracee.

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it contains an additional protein called apo(a) (12, 13). The majority of Lp(a) are present at densities between LDL and HDL (1.05–1.08 g/ml), but a small portion of plasma Lp(a) is found in the intermediate density lipoprotein (IDL) and VLDL density fractions. In this study, we call the former normal Lp(a) and the latter buoyant Lp(a). Bard et al. (14) estimated that about 20% of the Lp(a) particles contain apoE and are distributed in VLDL and IDL size range. Preliminary study revealed that the apoE-deficient subject in this study had a markedly increased level of normal Lp(a) and buoyant Lp(a), which contained apoB-100 or apoB-48 and apoA-IV. Fless et al. (15) have postulated that buoyant Lp(a) results from free apo(a) associating with TG-rich lipoproteins, and metabolism of the resultant Lp(a) depends on lipoprotein particles to which apo(a) binds.

To establish the role of apoE on the buoyant Lp(a) metabolism and, if so, how buoyant and normal Lp(a) are metabolically distinct, we investigated a series of in vivo kinetic studies where apoE-deficient buoyant Lp(a) and normal Lp(a) were injected into the apoE-deficient patient and the control subjects.

METHODS

Study subjects

One homozygous apoE-deficient patient subject and 10 control subjects were investigated in this study. The apoE-deficient subject was homozygous for the A to G substitution in the acceptor splice site of the third intron, causing aberrant splicing to produce two apoE mRNA containing either the entire or the 3′-portion of the third intron (16), and her characterization, including kinetic findings, were previously reported (10, 11, 17, 18). All study subjects had normal fasting plasma glucose levels and normal thyroid, hepatic, and renal function. All study subjects were hospitalized and 3 days prior to the study were placed on an isoweight diet containing 47% carbohydrate, 37% fat, 16% protein, 200 mg of cholesterol per 1,000 Kcal, and a polyunsaturated-saturated fat ratio of 0:3. Subjects gave written informed consent to the study protocols, which were approved by the Clinical Research Subpanel of the National Heart, Lung, and Blood Institute.

apoB-100 and apoB-48 kinetic study using stable isotopically-labeled tracer

After a 12 h fast, $^{13}C_6$-phenylalanine (99% $^{13}C_6$, Cambridge Isotope Laboratories, Woburn, MA) was administered to the homozygous apoE-deficient subject and seven control subjects (#1–#7) as a priming bolus of 600 μg/kg, immediately followed by a constant infusion of 12 μg/kg per minute for up to 12 h to 16 h. Blood samples (20 ml) were obtained from the opposite arm at 10 min, and at 1, 2, 3, 4, 5, 6, and every 2 h until the end of the infusion. In studies with the apoE-deficient subject and control subject #7, blood sampling continued at 18, 24, 36, 48, 72, 96, 120, and 168 h. During the infusion, meals were served in equal small portions every 2 h. Plasma was immediately separated by centrifugation at 2,300 rpm for 30 min at 4°C. VLDL, IDL, and LDL were isolated by sequential ultracentrifugation from 5 ml of plasma and processed for the analysis by gas chromatography-mass spectrometry (GC-MS) as previously described (19, 20). Briefly, lipoproteins were dialyzed against 10 mM ammonium bicarbonate, lyophilized, and delipidated. apoB-100 was isolated by preparative gradient NaDodSO4-PAGE (5–15%). Nonreducing PAGE was employed because the apoB-100 band was contaminated by Lp(a)-apoB-100 under reducing condition. apoB-100 and apoB-48 bands were cut from gels, subjected to hydrolysis in 6N HCl (Ultrapure grade, J.T. Baker, Inc., Phillipsburg, NJ) at 110°C for 24 h. The protein hydrolysates were lyophilized and free amino acids were purified by cation exchange chromatography (AG-50W-X8, Bio-Rad Laboratories, Richmond, CA). Recovered amino acids were derivatized to the N-heptafluorobutyryl isobutyl esters, dissolved in ethyl acetate, and analyzed by a Hewlett-Packard 5890/5988A, a GC-MS in the chemical ionization mode, using isobutane as the reagent gas. Selective ion monitoring at 418 *m/z* for unlabeled phenylalanine and 424 *m/z* for $^{13}C_6$-phenylalanine was used to determine the isotope ratio. Each sample was analyzed at least 2×. Acquired data were converted to tracer/tracee ratios using the method of Cobelli, Toffolo, and Foster (21).

A previously reported multicompartmental model (20) was built using an interactive computer program (SAAMII version 1.1, SAAM Institute, Inc., Seattle, WA) (22) to determine the apoB-100 kinetic parameters. In brief, the plasma phenylalanine pool was used as a forcing function (QL function), followed by a delay compartment for lipoprotein assembly and subsequent secretion from the liver. VLDL consisted of large and small compartments, accounting for the delipidation chain. A single compartment was allocated for IDL and LDL. As for a multicompartmental model for apoB-48 in the apoE-deficient subject, we first attempted to use the apoB-100 model, but could not get a reasonable fitting. We then modified an apoB-48 model structure as follows: *1)* a single VLDL apoB-48 compartment was used, and *2)* pathways from a large VLDL pool were replaced by direct synthetic pathways from the intestine to IDL and LDL apoB-48. We assumed that steady state conditions were maintained throughout the study period. Plasma volume was assumed to be 4% of body weight. Residence time (RT; days) was a reciprocal to the fractional catabolic rate (FCR; day^{-1}). Production rates (PR; mg/kg/d) were calculated as the products of FCR and pool.

Lp(a) kinetic study using radiolabeled tracer

Lp(a) particles were isolated from the fasting plasma of the apoE-deficient subject according to the procedure described by Fless, Rolih, and Scanu (23). Plasma was obtained after a 12 h fast, and NaEDTA (0.01%), sodium azide (0.05%), and difluorophosphonate (1 mM) were immediately added. Plasma was adjusted to a density of 1.21 g/ml using solid NaBr and ultracentrifuged for 48 h to isolate total plasma lipoproteins. The $d < 1.21$ g/ml fraction was adjusted to $d = 1.4$ g/ml with NaBr and ultracentrifuged on a 0–30% NaBr density gradient at 59,000 rpm for 2 h to remove HDL (bottom fraction). The fraction containing Lp(a) (top fraction) was then adjusted to a concentration of 7.5% CsCl and ultracentrifuged for 30 h to separate buoyant Lp(a) (top fraction) and normal Lp(a) (bottom fraction). Both fractions were subjected to chromatofocusing on a PBE94 column (Pharmacia, Uppsala, Sweden) to remove residual VLDL, IDL, and LDL. Isolated Lp(a) was analyzed for purity by nonreducing and reducing SDS-PAGE and by 0.6% agarose electrophoresis (Helena Laboratories, Beaumont, TX).

Lp(a) samples were extensively dialyzed against PBS with 0.01% EDTA before iodination using a modification of the iodine monochrolide method (24). Briefly, an equal volume of a 1 M glycine buffer was added to the Lp(a) sample. Five mCi ^{125}I or ^{131}I were then added to buoyant and normal Lp(a) solutions, followed by iodine monochrolide. Approximately 1 mol of iodine was incorporated per mole of Lp(a). Samples were dialyzed extensively against PBS/0.01% EDTA to remove free iodine. Human serum albumin was added to a final concentration of 5%

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TABLE 1. Characterization of study subjects

Subjects	Sex	Age	BMI	TC	TG	HDL-C	apoA-I	apoB	Lp(a)
		years	*kg/m²*			*mg/dl*			
apoE deficiency	F	68	27.8	442	171	69	163	92	87
Control subjects (n = 10)									
Mean		25.1	21.4	167.8	71.8	51.5	134.3	101.8	nd
SD		10.2	1.8	30.9	25.1	8.4	4.9	22.5	nd

apoE, apolipoprotein E; BMI, body mass index; HDL-C, HDL cholesterol; Lp(a), lipoprotein(a); nd, not determined; TC, total cholesterol; TG, triglyceride.

(wt/vol), and samples were sterile-filtered through a 0.22 μm filter and tested for pyrogens and sterility before injection. Iodinated Lp(a) particles were evaluated by SDS-PAGE and agarose electrophoresis.

Three days prior to injection, study subjects (the apoE-deficient subject and control subjects #8–10) were placed on an isoweight diet. Subjects were permitted to eat a normal diet but were instructed not to drink alcoholic beverages for 1 week before and during the study. One day before the injection, the subjects were given potassium iodide at a dose of 900 mg/d in divided doses, and this was continued for the duration of the study. ^{125}I-buoyant Lp(a) was injected into the apoE-deficient subject and control subject #8, and both ^{125}I-buoyant Lp(a) and ^{131}I-normal Lp(a) were injected into two control subjects (#9, #10) after a 12 h fast. Blood samples were obtained 10 min after injection, then at 1, 3, 6, 12, and 24 h, and at 2, 3, 4, 5, 7, 9, 11, and 14 days. Blood was drawn into the tubes containing EDTA at a final concentration of 0.1%, immediately placed at 4°C, and plasma was separated by low speed centrifugation in a refrigerated centrifuge. Sodium azide and aprotinin were added to the plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivity in the 4 ml plasma was quantitated in a Packard Cobra γ counter (Packard Instrument Co., Downers Grove, IL). Plasma curves were constructed by dividing the plasma radioactivity at each time point by the plasma activity at the initial 10 min point. Plasma radioactivity curves were fit by a multiexponential function using SAAMII. RTs were determined by the area under the curves.

Analytical methods

Total cholesterol and TG levels were determined by automated enzymatic techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol (HDL-C) was measured by dextran sulfate precipitation (25). apoB concentration was measured by competitive enzyme-linked immunoassay (26). apoB-100 and apoB-48 bands on NaDodSO4-PAGE gels were quantitated using a laser densitometer (BioRad, Richmond, CA).

RESULTS

Subjects

Characterization of the study subjects is shown in **Table 1**. The apoE-deficient subject is a 68-year-old female who has been previously described (10). The plasma cholesterol level of 442 mg/dl was disproportionately higher than the TG level of 171 mg/dl. The VLDL-cholesterol/TG ratio was increased considerably to 0.94 in this patient (0.96 as the mean of the other eight homozygous patients), as compared with a normal value of 0.2, demonstrating the accumulation of remnants. Despite high cholesterol and TG levels, HDL-C and its major apolipoprotein constituent, apoA-I, were not low but elevated to 69 mg/dl and 163 mg/dl. She developed a documented coronary artery disease at the age of 57 years. Coronary artery disease was also evident in two other homozygotes (both siblings), her father, and two paternal uncles, thus, although the onsets were not markedly premature, demonstrating a strong segregation of coronary atherosclerosis in these kindred. Plasma Lp(a) concentration in her plasma was markedly increased to 87 mg/dl. Her Lp(a) phenotype was determined by an immunoblot demonstrated S1/S4 heterozygote.

Nonreducing SDS-PAGE of VLDL, IDL, and LDL in the apoE-deficient subject is shown in **Fig. 1**. apoB-48 is present in all apoB-containing lipoprotein fractions, including LDL. apoB-48/apoB-100 ratios quantified by a laser densitometer were 0.360 in VLDL, 0.199 in IDL, and 0.181 in LDL, respectively. In contrast to the typical Lp(a) distribution pattern, where Lp(a) is almost exclusively present between LDL and HDL density range, a significant portion of Lp(a), a mainly smaller isoform of Lp(a) called S1, exists in VLDL and IDL. In this study, we designate the former Lp(a) as normal Lp(a) and the latter as buoyant Lp(a).

apoB-100 metabolism

Tracer/tracee (T/T) ratios of apoB-100 in VLDL, IDL, and LDL in the apoE-deficient subject are shown in **Fig. 2A**. T/T ratios were fit by the multicompartmental model

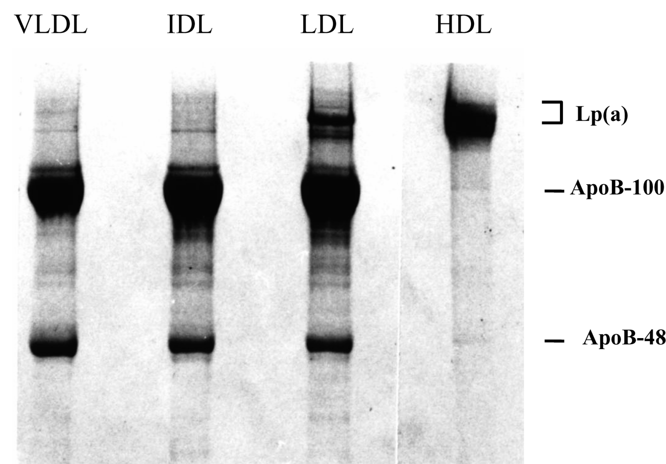

Fig. 1. Nonreducing SDS-PAGE of lipoproteins from the patient with apolipoprotein E (apoE) deficiency. IDL, intermediate density lipoprotein; Lp(a), lipoprotein(a).

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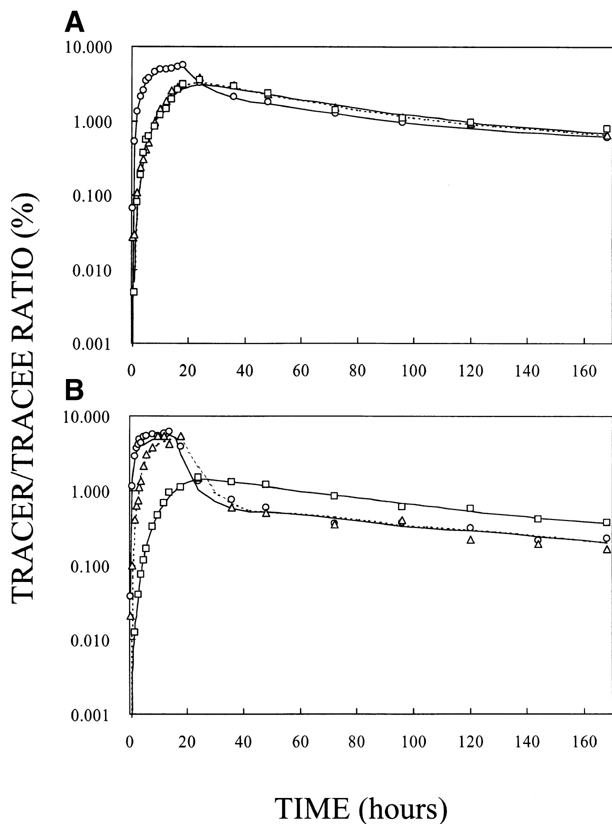

Fig. 2. Tracer/tracee ratio curves for VLDL apoB-100 (open circles with solid line), IDL apoB-100 (open triangles with dotted line), and LDL apoB-100 (open squares with solid line) in the apoE-deficient subject (A) and the representative control subject #7 (B). Data were fitted by the multicompartmental model using SAAMII.

to determine the kinetic parameters. VLDL apoB-100 T/T ratio increased slowly during the constant infusion and then decayed slowly during the washout phase relative to those in a representative control subject (Fig. 2B). A substantial difference was observed in the IDL apoB-100 T/T curve. In a control subject, the IDL T/T ratio increased relatively rapidly to follow the VLDL apoB-100 T/T curve and then rapidly decayed after the termination of the infusion (Fig. 2B). In contrast, the IDL T/T ratio increased and decayed very slowly during and after the infusion period in the apoE-deficient subject (Fig. 2A), whereby the IDL apoB-100 T/T is almost indistinguishable from the LDL apoB-100 T/T curve. The LDL apoB-100 T/T curve, however, increased more rapidly during the infusion and decreased more rapidly during the washout phase in the apoE-deficient subject than in the control subject.

Kinetic parameters determined by the multicompartmental model are listed in **Table 2**. The FCR of VLDL apoB-100 in the apoE-deficient subject was decreased by 68% to 3.75 pools/d as compared with control subjects (11.65 ± 7.57 pools/d). Furthermore, the IDL apoB-100 FCR was more severely impaired, with 1.20 pools/d in the apoE-deficient subject. The FCR of LDL apoB-100 was 1.07 pools/d, similar to IDL apoB-100 FCR but increased by about 3-fold as compared with control subjects. The rates of production of apoB-100 in the apoE-deficient patient were decreased by 45% in VLDL and 78% in IDL but remained unchanged in LDL. Thus, the increased steady state apoB-100 levels in VLDL (54%) and IDL (137%) resulted primarily from markedly decreased FCR and were present despite the decreased PR. In contrast, the decreased LDL apoB level (−67%) was solely due to the accelerated catabolism in the apoE-deficient subject.

Detailed kinetic information obtained by multicompartmental analysis is summarized in **Table 3** and **Fig. 3A**. apoE deficiency resulted in essentially no direct removal of small VLDL and IDL (Fig. 3A), thus removal of VLDL and IDL accounts for only 24% of total removal, about one-third of that in control subjects, where 63% of apoB-containing particles are removed as VLDL or IDL (Table 3). Rate constants for lipolytic pathways from large VLDL to LDL via small VLDL and IDL were markedly decreased by 84% to 96% or 0.79 pools/d (small VLDL to IDL) to 1.20 pools/d (IDL to LDL), as compared with control subjects (Fig. 3A). Although conversion pathways were slowed, the percentage of VLDL converted into LDL was increased to 76% as compared with 37% of the mean of control subjects, mainly due to the complete block of the apoE-mediated removal pathways of VLDL and IDL (Ta-

TABLE 2. Kinetic parameters of apoB-100 and apoB-48 in the patient with apoE deficiency

	VLDL			IDL			LDL		
	conc	FCR	PR	conc	FCR	PR	conc	FCR	PR
	mg/dl	*day⁻¹*	*mg/kg/d*	*mg/dl*	*day⁻¹*	*mg/kg-d*	*mg/dl*	*day⁻¹*	*mg/kg-d*
apoB-100									
apoE deficiency	12.7	3.75	19.0	11.6	1.20	5.6	33.4	1.07	14.3
Control subjects (n = 7)									
Mean	8.2	11.65	34.8	4.9	13.98	25.7	100.5	0.41	16.5
SD	3.5	7.57	18.1	1.5	7.48	12.3	8.7	0.11	5.2
apoB-48									
apoE deficiency	7.56	0.59	1.78	4.47	1.35	2.42	11.62	0.78	3.61
Control subjects (n = 4)									
Mean	6.96								
SD	1.63								

conc, concentration; FCR, fractional catabolic rate; IDL, intermediate density lipoprotein; PR, production rate.

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TABLE 3. Metabolic channeling of apoB-100 in the patient with apoE deficiency

	Removal from			Conversion from VLDL to		LDL Synthesis	
	VLDL	IDL	LDL	IDL	LDL	Shunt Pathway	Cascade
				%			
apoE deficiency	24.4	0.0	75.6	29.3	75.6	61.3	38.7
Control subjects (n = 7)							
Mean	44.4	18.9	36.7	51.7	36.7	12.7	87.3
SD	17.3	21.7	22.3	17.2	22.3	10.9	10.9

ble 3). The LDL production pathway was altered by apoE deficiency: 61% of LDLs were directly produced from large VLDL via a shunt pathway, whereas this pathway accounted for only 13% of LDL production in control subjects (Table 3).

apoB-48 metabolism

The T/T ratios of apoB-48 in VLDL, IDL, and LDL in the apoE-deficient subject are shown in **Fig. 4A**. The rate of increase during the infusion and decay in the washout phase was markedly decreased in VLDL apoB-48 as compared with the VLDL apoB-48 curve in a representative control subject (Fig. 4B), and, again, IDL and LDL apoB-48 tracer/tracee ratios showed similar curves in the apoE-deficient patient. The IDL and LDL apoB-48 T/T ratios were not measured in the control subjects due to their trace amounts. The kinetic parameters of apoB-48 are summarized in Table 2. VLDL apoB-48 FCR was markedly decreased by 92%, or 0.59 pools/d, as compared with the average of four control subjects of 6.96 ± 1.63 pools/d. Furthermore, the apoB-48 FCRs in IDL and LDL were also low and similar to the apoB-100 FCR counterpart. With regard to the VLDL apoB-100/apoB-48 FCR ratio, it was 6.96 in the apoE-deficient subject as compared with 1.85 ± 1.08 pools/d in control subjects, demonstrating that apoE deficiency resulted in a more striking delay of VLDL apoB-48 catabolism relative to VLDL apoB-100 catabolism. As shown in Fig. 3B, this impaired VLDL apoB-48 catabolism was due to the absence of apoE-mediated uptake along with the impaired conversion pathway (0.59 pools/d). Direct removal of IDL apoB-48 was also completely blocked, thus indicating that apoE play a pivotal role on the receptor-mediated uptake of VLDL/chylomicron and their remnants. Interestingly, in addition to VLDL apoB-

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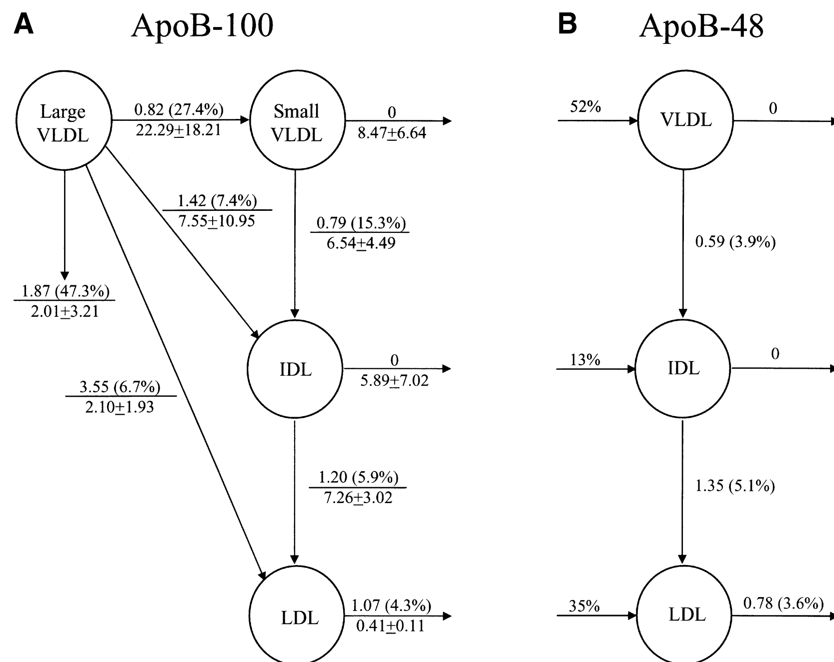

Fig. 3. Rate constants of apoB-100 (A) and apoB-48 (B) in the apoE-deficient subject. A: Values above the line represent rate constants (fractional standard deviation %) in the apoE-deficient subject, and those below the lines represent the mean rate constant ± SD of seven control subjects. Unit for rate constant is given as pools/day. B: Rate constants of apoB-48 in the apoE-deficient subject. Rate constants are given as the best estimate (fractional standard deviation percent). Percentages listed on the left side above the lines represent relative distribution of lipoproteins secreted from the intestine.

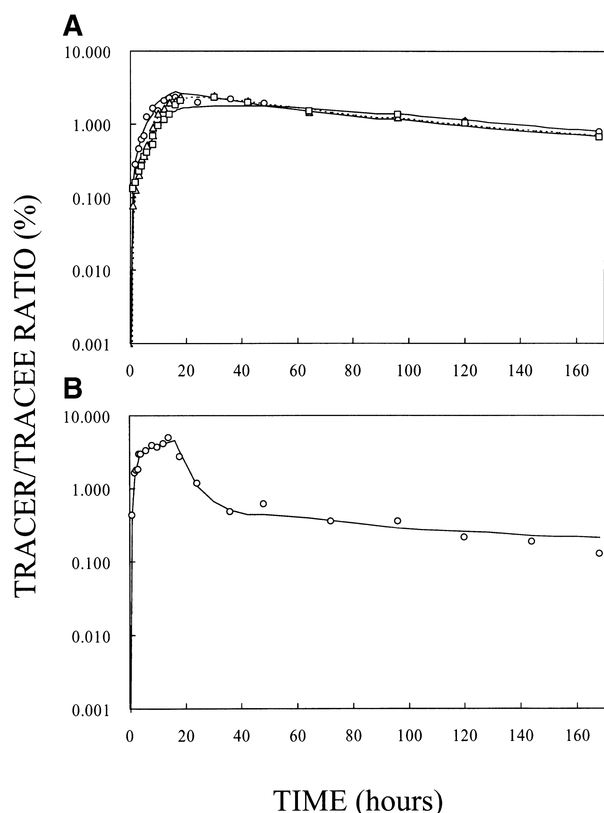

Fig. 4. Tracer/tracee ratio curves for VLDL apoB-48 (open circles with solid line), IDL apoB-48 (open triangles with dotted line), and LDL apoB-48 (open squares with solid line) in the apoE-deficient subject (A), and the tracer/tracee ratio curve for VLDL apoB-48 in the representative control subject #7 (B). Data were fitted by the multicompartmental model using SAAMII.

48, the intestine directly secreted IDL apoB-48 (13%) and LDL apoB-48 (35%), showing that newly secreted apoB-48 containing lipoproteins are heterogeneous in density.

Lp(a) metabolism

Characterization of buoyant and normal Lp(a) isolated from the apoE-deficient subject is shown in **Fig. 5**. Autoradiography of buoyant and normal Lp(a) particles demonstrated a preβ migration on agarose gel electrophoresis (Fig. 5A). Furthermore, as shown in Fig. 5B, buoyant Lp(a) is primarily comprised of small size Lp(a) [Lp(a)-S1], whereas normal Lp(a) comprised both large [Lp(a)-S4] and small Lp(a) [Lp(a)-S1]. Plasma radioactivity decay curves of ^{125}I-buoyant Lp(a) and the RT in the apoE-deficient patient and three control subjects (#8–10) are shown in **Fig. 6** and **Table 4**. Buoyant Lp(a) isolated from the apoE-deficient patient was catabolized almost twice as slowly in the apoE-deficient subject (3.85 days) than in the control subjects (2.15 ± 0.33 days). We then compared metabolism of ^{125}I-buoyant and ^{131}I-normal Lp(a) isolated from the apoE-deficient patient in three control subjects (#8–10) for the former and two control subjects (#9, 10) for the latter. As shown in **Fig. 7** and Table 4, ^{125}I-buoyant Lp(a) (2.15 ± 0.33 days) were catabolized at twice as fast as ^{131}I-normal Lp(a) (4.23 ± 0.20 days) in the control subjects. Interestingly, the mean RT of apoE-deficient-normal Lp(a) was not different from that of normal Lp(a) isolated from a control subject (3.51 ± 0.31 days) (27), suggesting that normal Lp(a) is catabolized in apoE-independent manner.

DISCUSSION

apoE deficiency is a rare genetic disease characterized by palmer and tuboeruptive xanthomas, severe type III hyperlipidemia, and, in some cases, premature atherosclerotic vascular disease. To date, five kindreds (10, 16–18, 28–32) have been reported. The present study was designed to further extend the knowledge gained from two previous kinetic studies in apoE deficiency (10, 11). An exogenous radiotracer LDL kinetic study in the same patient revealed an increased catabolism of LDL probably due to upregulation of LDL receptor activity (11). However, the LDL isolated from her plasma by ultracentrifugation almost certainly contained substantial Lp(a). In fact, this is consistent with the finding that LDL from apoE deficiency was catabolized at slower rates than autologous LDL in normal subjects, based on the fact that Lp(a) is catabolized at a slower rate than LDL apoB-100 (27). In another study, where radiolabeled apoE-deficient VLDL was used as a tracer, catabolism of VLDL apoB-100 and B-48 were found to be severely impaired. However, the lack of analysis using a multicompartmental model did not permit a comprehensive assessment of apoB-containing lipoprotein metabolism.

We attempted perform a series of kinetic studies to explore underlying metabolic abnormalities of apoB-100 and apoB-48 containing lipoproteins as well as Lp(a) in human apoE deficiency. We employed an endogenous labeling technique using stable, isotopically-labeled amino acid and muticompartmental modeling analysis in order to conclude the in vivo effect of apoE deficiency on apoB metabolism. We found that apoE deficiency resulted in elimination of direct catabolism of VLDL and IDL, as well as an impaired conversion to LDL. Both metabolic alternations delayed the rate of catabolism of apoB, in particular, apoB-48. Factors regulating catabolism are receptor-mediated removal and lipolysis, thus, the markedly impaired catabolism of the apoB-100- and apoB-48-containing VLDL/IDL in apoE deficiency clearly demonstrates that apoE play a critical role in vivo for these processes. In particular, we showed that IDL apoB-100 and VLDL apoB-48 were catabolized 12× more slowly than controls, indicating that apoE is a major metabolic determinant for these particles.

On the other hand, the LDL apoB-100 catabolic rate was increased in the apoE-deficient subject by nearly 3-fold. Given that LDL particles from apoE patients have a lower affinity to the LDL-receptor (11), it is likely that the LDL receptor activity is upregulated in the apoE-deficient subject. Previous animal studies (33, 34) demonstrated that apoE-containing lipoproteins can effectively compete with LDL for LDL receptor-mediated uptake. Inasmuch as

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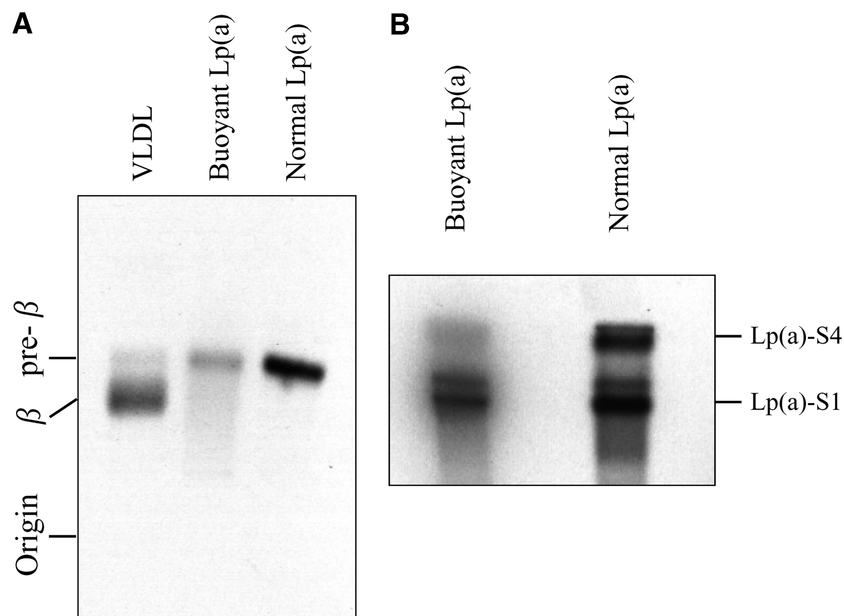

Fig. 5. Autoradiographs of buoyant and normal Lp(a) isolated from the apoE-deficient subject. A: Agarose gel electrophoresis of buoyant and normal Lp(a), together with VLDL isolated from the apoE-deficient subject. Both buoyant and normal Lp(a) migrate at preβ position, whereas VLDL migrates at β position. B: Nonreducing NaDodSO4-PAGE of buoyant and normal Lp(a). Lp(a) comprises two isoforms: Lp(a)-S4 for the large isoform and Lp(a)-S1 for the small isoform.

apoE-deficient VLDL/IDL cannot compete with LDL for an LDL receptor binding site, LDL is removed more effectively through this pathway. This process may be further accelerated by decreased hepatic cholesterol content, caused by poor delivery of apoE-deficient VLDL/IDL cholesterol to the liver, leading to increased hepatic LDL receptor activity. To our knowledge, apoE deficiency is the first case where IDL and LDL apoB are catabolized at similar rates.

In contrast to the complete elimination of direct removal for small VLDL and IDL, we found a "normal" removal rate of large VLDL in the apoE-deficient subject. Supporting this surprising finding is a study by Tomiyasu et al. (35), which showed an increased direct removal of large VLDL without apoE as compared with those with apoE. By electron microscopy, VLDL in apoE-deficient subjects are more heterogeneous in size as compared with "classic" type III hyperlipidemia or healthy controls (10, 31, 36). Inhibition of direct removal of IDL apoB-100 is supported by a kinetic study by Turner et al. (37) using dyslipidemic E2 homozygotes, but not by the other study by Demant et al. (38) using normolipidemic E2 homozygotes. Although the exact reason for the discrepancy is not clear, it is possible that direct removal of IDL is more profoundly impaired by the lack of apoE or the presence of dysfunctional apoE with dyslipidemia as compared with dysfunctional apoE but with normal lipids. The catabolic rate of LDL apoB-48 in the apoE deficiency was 0.78 pools/day. No kinetic data for LDL apoB-48 are available in other studies for comparison with how LDL apoB-48 was removed from the circulation of our patient. apoE has been considered as a crucial ligand for the removal of chylomicron remnants when removed via LRP or VLDL receptors (39), thus these receptors being unlikely candidates for the removal. A newly isolated receptor, apoB-48 receptor (40), may have potential, but its significance in chylomicron metabolism in humans has yet to be established. The interaction of candidate receptors with apoE-deficient LDL apoB-48 will be a key study to clarify this question.

Another important observation in the present study was markedly decreased hepatic VLDL production in the apoE-deficient subject. The exact mechanism for the decreased VLDL production is not clear at present. apoE-

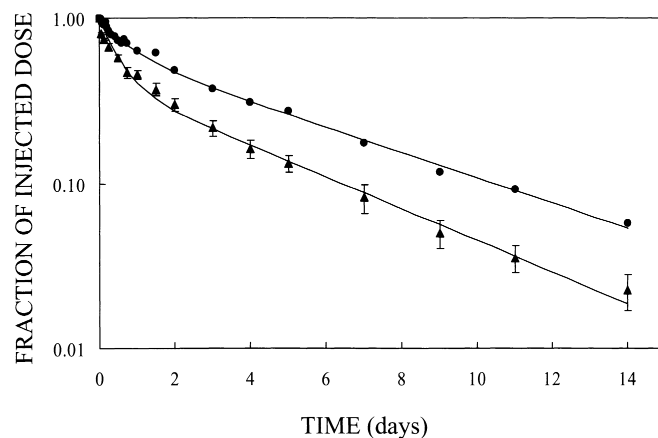

Fig. 6. Metabolism of ^{125}I-buoyant Lp(a) isolated from the apoE-deficient subject in the apoE-deficient subject (closed circles) and in the control subjects (closed triangles). Data from the control subjects are given as the mean ± SE.

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TABLE 4. Residence time of Lp(a)

	Buoyant Lp(a)	Normal Lp(a)	Normal Lp(a)
	from the apoE-Deficient Patient		from a Control
	days		
apoE deficiency	3.85		
Control subjects			
#8	1.75		
#9	2.56	4.03	
#10	2.13	4.42	
Mean	2.15	4.23	3.51[a]
SD	0.33	0.20	0.31[a]

[a] Data from Rader et al. (27).

deficient mice have been shown to have an impaired VLDL-TG secretion (41). We (7, 8) and others (5, 42, 43) reported that apoE gene transfer promoted VLDL TG and apoB secretion in this animal model. Indeed, Demant et al. (38) reported that VLDL apoB synthesis was significantly decreased in subjects with dysfunctional apoE (apoE2) as compared with those with normal apoE (apoE3), an in vivo evidence that apoE functionality associates with VLDL production. Overall, the present study supports the notion that apoE may facilitate hepatic VLDL secretion in humans.

The production and origin of IDL and LDL were also affected by apoE deficiency. apoB-100s containing IDL and LDL are more dependent on large VLDL as their origin in the apoE-deficient subject than in control subjects. This pathway was reported first in homozygous FH (44), then in other types of hyperlipidemia (45, 46), and also in normal subjects (47) and help fit "early" rise of tracer/tracee ratio of IDL and LDL apoB-100 as was observed in our apoE-deficient subject. Based on modeling studies by Fisher (48) and Beltz (49), this pathway is proposed to represent a rapid and complete lipolysis, resulting in conversion to LDL or direct removal by LDL receptor. In our opinion, the finding that VLDL in subjects with apoE deficiency, including our subject, are heterogeneous in size (10, 31) is consistent with the current compartmental model, and it is possible that large VLDL has a distinct metabolism as compared with small VLDL, which is only slowly converted to IDL with no direct removal. However, the exact mechanisms accounting for this pathway remains unclear. IDL apoB production was found to be only one-fifth of normal production. This finding, together with the finding that IDL input from small VLDL through lipoprotein lipase-mediated lipolysis was markedly impaired, indicates lipolysis reaction as a major metabolic determinant for IDL production.

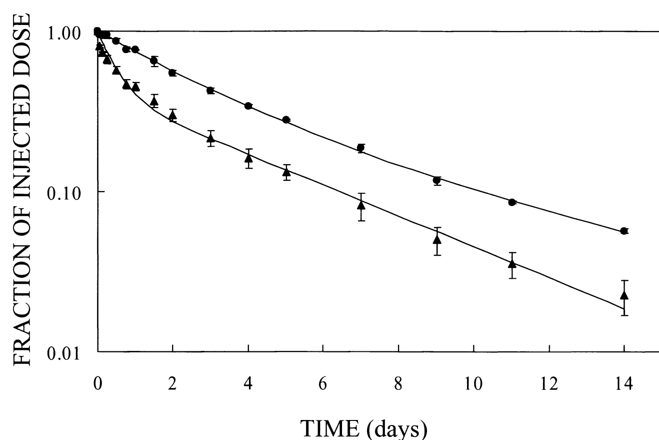

Fig. 7. Metabolism of ^{125}I-buoyant Lp(a) (closed triangles) and ^{131}I-normal Lp(a) (closed circles) isolated from the apoE-deficient subject in the control subjects. Data from the control subjects are given as the mean ± SE.

Human apoE deficiency provides us an unique opportunity to assess the effect of apoE deficiency on Lp(a) metabolism. In the apoE-deficient subject, in addition to "normal" Lp(a), we identified "buoyant" Lp(a), which existed within VLDL and IDL density range and contained apoB-48, apoA-IV, and apoCs. It's been shown that apo(a) is not only found at the densities between 1.055 to 1.12 g/ml but can be associated with TG-rich lipoproteins, in particular, postprandially (50, 51). This "postprandial" Lp(a) contains apoE, more TG and is distributed mainly in VLDL and IDL density range. Therefore, it is conceivable that "buoyant" Lp(a) found in the apoE-deficient subject is the product of free apo(a) associating with chylomicron and VLDL and their remnants.

Buoyant Lp(a) from the apoE-deficient subject was labeled and injected into apoE-deficient subject and control subjects to assess how association of apoE with buoyant Lp(a) modulates buoyant Lp(a) metabolism. We found that buoyant Lp(a) was catabolized at a slower rate than in control subjects. Based on the easily-transferable feature of apoE, this finding can be interpreted as indicating that, in control subjects, injected apoE-deficient buoyant Lp(a) acquired apoE to accelerate its catabolism. To our knowledge, this is the first demonstration that apoE can modulate Lp(a) metabolism in vivo. We differentially labeled and injected buoyant and normal Lp(a) isolated from the apoE-deficient subjects into the normal subjects to answer the second question of whether metabolism of buoyant and normal Lp(a) differ. We found that buoyant Lp(a) was catabolized at a faster rate than normal Lp(a) in the control subjects. We also observed radioactivity of buoyant Lp(a) in densities where normal Lp(a) resided, indicating that buoyant Lp(a) was converted to normal Lp(a). Taken together, we suggest that buoyant Lp(a) is a precursor for normal Lp(a), and apoE plays an essential role to convert buoyant Lp(a) into normal Lp(a). Of note is the fact that the apoE-deficient normal Lp(a) was catabolized at a similar rate to the normal Lp(a) isolated from the control subjects (27), indicating that once mature Lp(a) has been formed, the subsequent metabolism of Lp(a) is apoE-independent. However, it should be cautious to extend this observation to normolipidemic humans, because there are very trace amounts, if any, of "buoyant" Lp(a) in normolipidemic subjects.

The present study has some limitations. First, we only studied one apoE-deficient subject. In this regard, we have a theoretical limitation to generalize the findings to other

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apoE-deficient patients. However, all but one apoE-deficient subject, with poorly controlled diabetes and heavy drinking (29), show similar lipid profiles. We, therefore, believe that the results from this single patient with this very rare genetic disease are generalizeable and provide important insights into the role of apoE in lipoprotein metabolism.

In summary, apoE deficiency in humans results in a markedly impaired catabolism of VLDL/chylomicron and their remnants, an increased rate of catabolism of LDL apoB-100, reduced VLDL apoB production, and delayed catabolism of buoyant Lp(a). This study provides new insights into the role of apoE in apoB and Lp(a) metabolism and indicates that delivery of apoE may be a novel method to reverse proatherogenic lipoprotein profile by reducing atherogenic remnants and Lp(a).

The authors are indebted to Marie Kindt, Glenda Tally, Susan Meyn, and Yoshiko Doherty for excellent technical support. The authors thank George Grimes and the Pharmaceutical Development Service for the pyrogen test of injected materials and Betty Kuzmik and the nursing staff of the National Institutes of Health Clinical Center for nursing care of the study subjects.

REFERENCES

1. Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* **240:** 622–630.
2. Mahley, R. W., Y. Huang, and S. C. Rall, Jr. 1999. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. *J. Lipid Res.* **40:** 1933–1949.
3. Mahley, R. W., and Y. Huang. 1999. Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol.* **10:** 207–217.
4. Huang, Y., X. Q. Liu, S. C. Rall, Jr., and R. W. Mahley. 1998. Apolipoprotein E2 reduces the low density lipoprotein level in transgenic mice by impairing lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins. *J. Biol. Chem.* **273:** 17483–17490.
5. Huang, Y., X. Q. Liu, S. C. Rall, Jr., J. M. Taylor, A. von Eckardstein, G. Assmann, and R. W. Mahley. 1998. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. *J. Biol. Chem.* **273:** 26388–26393.
6. Thuren, T., K. H. Weisgraber, P. Sission, and M. Waite. 1992. Role of apolipoprotein E in hepatic lipase catalyzed hydrolysis of phospholipid in high-density lipoproteins. *Biochemistry.* **31:** 2332–2338.
7. Tsukamoto, K., C. Maugeais, J. M. Glick, and D. J. Rader. 2000. Markedly increased secretion of VLDL triglycerides induced by gene transfer of apolipoprotein E isoforms in apoE-deficient mice. *J. Lipid Res.* **41:** 253–259.
8. Maugeais, C., U. J. Tietge, K. Tsukamoto, J. M. Glick, and D. J. Rader. 2000. Hepatic apolipoprotein E expression promotes very low density lipoprotein-apolipoprotein B production in vivo in mice. *J. Lipid Res.* **41:** 1673–1679.
9. Plump, A. S., and J. L. Breslow. 1995. Apolipoprotein E and the apolipoprotein E-deficient mouse. *Annu. Rev. Nutr.* **15:** 495–518.
10. Schaefer, E. J., R. E. Gregg, G. Ghiselli, T. M. Forte, J. M. Ordovas, L. A. Zech, and H. B. Brewer, Jr. 1986. Familial apolipoprotein E deficiency. *J. Clin. Invest.* **78:** 1206–1219.
11. Gabelli, C., R. E. Gregg, L. A. Zech, E. Manzato, and H. B. Brewer, Jr. 1986. Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency. *J. Lipid Res.* **27:** 326–333.
12. Utermann, G. 1989. The mysteries of lipoprotein(a). *Science.* **246:** 904–910.
13. Scanu, A. M., and G. M. Fless. 1990. Lipoprotein(a): heterogeneity and biological relevance. *J. Clin. Invest.* **85:** 1709–1715.
14. Bard, J. M., S. Delattre-Lestavel, V. Clavey, P. Pont, B. Derudas, H. J. Parra, and J. C. Fruchart. 1992. Isolation and characterization of two sub-species of Lp(a), one containing apoE and one free of apoE. *Biochim. Biophys. Acta.* **1127:** 124–130.
15. Fless, G. M., D. Pfaffinger, J. D. Eisenbart, and A. M. Scanu. 1990. Solubility, immunochemical, and lipoprotein binding properties of apoB100-apo(a), the protein moiety of lipoprotein(a). *J. Lipid Res.* **31:** 909–918.
16. Cladaras, C., M. Hadzopoulou-Cladaras, B. K. Felber, G. Pavlakis, and V. I. Zannis. 1987. The molecular basis of a familial apoE deficiency. An acceptor splice site mutation in the third intron of the deficient apoE gene. *J. Biol. Chem.* **262:** 2310–2315.
17. Ghiselli, G. E., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1241.
18. Anchors, J. M., R. E. Gregg, S. W. Law, and H. B. Brewer, Jr. 1986. ApoE deficiency: markedly decreased levels of cellular apoE mRNA. *Biochem. Biophys. Res. Commun.* **134:** 937–943.
19. Ikewaki, K., D. J. Rader, T. Sakamoto, M. Nishiwaki, N. Wakimoto, J. R. Schaefer, T. Ishikawa, T. Fairwell, L. A. Zech, H. Nakamura, M. Nagano, and H. B. Brewer. 1993. Delayed catabolism of high density lipoprotein apolipoprotein A-I and A-II in human cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **92:** 1650–1658.
20. Ikewaki, K., M. Nishiwaki, T. Sakamoto, T. Ishikawa, T. Fairwell, L. A. Zech, M. Nagano, H. Nakamura, H. B. Brewer, Jr., and D. J. Rader. 1995. Increased catabolic rate of low density lipoproteins in humans with cholesteryl ester transfer protein deficiency. *J. Clin. Invest.* **96:** 1573–1581.
21. Cobelli, C., G. Toffolo, and D. M. Foster. 1992. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** E968–E975.
22. SAAMII. 1994. SAAMII User Guide. University of Washington. Seattle, Washington.
23. Fless, G. M., C. A. Rolih, and A. M. Scanu. 1984. Heterogeneity of human plasma apolipoprotein(a). Isolation and characterization of the lipoprotein subspecies and their apoproteins. *J. Biol. Chem.* **259:** 11470–11478.
24. Rader, D. J., W. Cain, L. A. Zech, D. Usher, and H. B. Brewer, Jr. 1993. Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein (a) isoform is determined by the rate of lipoprotein(a) production. *J. Clin. Invest.* **91:** 443–447.
25. Warnick, G. R., M. C. Cheung, and J. J. Albers. 1979. Comparison of current methods for high-density lipoprotein cholesterol quantitation. *Clin. Chem.* **25:** 596–604.
26. Bojanovski, M., R. E. Gregg, D. M. Wilson, and H. B. Brewer, Jr. 1987. Semi-automated enzyme-linked immunosorbent assay (ELISA) for the quantification of apolipoprotein B using monoclonal antibodies. *Clin. Chim. Acta.* **170:** 271–280.
27. Rader, D. J., W. A. Mann, W. Cain, H. G. Kraft, D. Usher, L. A. Zech, J. M. Hoeg, J. Davignon, P. Lupien, M. Grossman, J. M. Wilson, and H. B. J. Brewer. 1995. The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. *J. Clin. Invest.* **95:** 1403–1408.
28. Mabuchi, H., H. Itoh, M. Takeda, K. Kajinami, T. Wakasugi, J. Koizumi, R. Takeda, and C. Asagami. 1989. A young type III hyperlipoproteinemic patient associated with apolipoprotein E deficiency. *Metabolism.* **38:** 115–119.
29. Kurosaka, D., T. Teramoto, T. Matsushima, T. Yokoyama, A. Yamada, T. Aikawa, Y. Miyamoto, and K. Kurokawa. 1991. Apolipoprotein E deficiency with a depressed mRNA of normal size. *Atherosclerosis.* **88:** 15–20.
30. Lohse, P., H. B. Brewer III, M. S. Meng, S. I. Skarlatos, J. C. LaRosa, and H. B. Brewer, Jr. 1992. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. *J. Lipid Res.* **33:** 1583–1590.
31. Feussner, G. 1996. Severe xanthomatosis associated with familial apolipoprotein E deficiency. *J. Clin. Pathol.* **49:** 985–989.
32. Feussner, G., J. Dobmeyer, H. J. Grone, S. Lohmer, and S. Wohlfeil. 1996. A 10-bp deletion in the apolipoprotein epsilon gene causing apolipoprotein E deficiency and severe type III hyperlipoproteinemia. *Am. J. Hum. Genet.* **58:** 281–291.
33. Woollett, L., Y. Osono, J. Herz, and J. M. Dietschy. 1995. Apolipoprotein E competitively inhibits receptor-dependent low density lipoprotein uptake by the liver but has no effect on cholesterol absorption or synthesis in the mouse. *Proc. Natl. Acad. Sci. USA.* **92:** 12500–12504.
34. Fan, J., Z. S. Ji, Y. Huang, H. de Silva, D. Sanan, R. W. Mahley, T. L. Innerarity, and J. M. Taylor. 1998. Increased expression of apolipo-

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protein E in transgenic rabbits results in reduced levels of very low density lipoproteins and an accumulation of low density lipoproteins in plasma. *J. Clin. Invest.* **101:** 2151–2164.
35. Tomiyasu, K., B. W. Walsh, K. Ikewaki, H. Judge, and F. M. Sacks. 2001. Differential metabolism of human VLDL according to content of ApoE and ApoC-III. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1494–1500.
36. Teramoto, T., M. Kinoshita, M. Kawamura, Y. Q. Liang, K. Kaneko, N. Shimazu, S. Suga, N. Akatuka, and M. Yamanaka. 1996. Characterization of low-density-lipoprotein in apolipoprotein E deficiency in a patient without coronary atherosclerosis. *Clin. Chim. Acta.* **247:** 159–166.
37. Turner, P. R., C. Cortese, R. Wootton, C. Marenah, N. E. Miller, and B. Lewis. 1985. Plasma apolipoprotein B metabolism in familial type III dysbetalipoprpteinemia. *Eur. J. Clin. Invest.* **15:** 100–112.
38. Demant, T., D. Bedford, C. J. Packard, and J. Shephard. 1991. Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. *J. Clin. Invest.* **88:** 1490–1501.
39. Herz, J., and D. K. Strickland. 2001. LRP: a multifunctional scavenger and signaling receptor. *J. Clin. Invest.* **108:** 779–784.
40. Brown, M. L., M. P. Ramprasad, P. K. Umeda, A. Tanaka, Y. Kobayashi, T. Watanabe, H. Shimoyamada, W. L. Kuo, R. Li, R. Song, W. A. Bradley, and S. H. Gianturco. 2000. A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **97:** 7488–7493.
41. Kuipers, F., M. C. Jong, Y. Lin, M. Eck, R. Havinga, V. Bloks, H. J. Verkade, M. H. Hofker, H. Moshage, T. J. Berkel, R. J. Vonk, and L. M. Havekes. 1997. Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E-deficient mouse hepatocytes. *J. Clin. Invest.* **100:** 2915–2922.
42. Huang, Y., Z. S. Ji, W. J. Brecht, S. C. Rall, Jr., J. M. Taylor, and R. W. Mahley. 1999. Overexpression of apolipoprotein E3 in transgenic rabbits causes combined hyperlipidemia by stimulating hepatic VLDL production and impairing VLDL lipolysis. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2952–2959.
43. Mensenkamp, A. R., M. C. Jong, H. van Goor, M. J. van Luyn, V. Bloks, R. Havinga, P. J. Voshol, M. H. Hofker, K. W. van Dijk, L. M. Havekes, and F. Kuipers. 1999. Apolipoprotein E participates in the regulation of very low density lipoprotein-triglyceride secretion by the liver. *J. Biol. Chem.* **274:** 35711–35718.
44. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very-low-and low-density lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **28:** 247–256.
45. Janus, E. D., A. Nicoll, R. Wootton, P. R. Turner, P. J. Magill, and B. Lewis. 1980. Quantitative studies of very low density lipoprotein: conversion to low density lipoprotein in normal controls and primary hyperlipidaemic states and the role of direct secretion of low density lipoprotein in heterozygous familial hypercholesterolaemia. *Eur. J. Clin. Invest.* **10:** 149–159.
46. Egusa, G., W. F. Beltz, S. M. Grundy, and B. V. Howard. 1985. Influence of obesity on the metabolism of apolipoprotein B in humans. *J. Clin. Invest.* **76:** 596–603.
47. Kesaniemi, Y. A., W. F. Beltz, and S. M. Grundy. 1985. Comparisons of metabolism of apolipoprotein B in normal subjects, obese patients, and patients with coronary heart disease. *J. Clin. Invest.* **76:** 586–595.
48. Fisher, W. R., L. A. Zech, L. L. Kilgore, and P. W. Stacpoole. 1991. Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [3H]leucine tracer. *J. Lipid Res.* **32:** 1823–1836.
49. Beltz, W. F., Y. A. Kesaniemi, B. V. Howard, and S. M. Grundy. 1985. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, intermediate density lipoproteins, and low density lipoproteins. *J. Clin. Invest.* **76:** 575–585.
50. McConathy, W. J., V. N. Trieu, E. Koren, C. S. Wang, and C. C. Corder. 1994. Triglyceride-rich lipoprotein interactions with Lp(a). *Chem. Phys. Lipids.* **67–68:** 105–113.
51. Scanu, A. M., D. Pfaffinger, and C. Edelstein. 1994. Post-prandial Lp(a): identification of a triglyceride-rich particle containing apoE. *Chem. Phys. Lipids.* **67–68:** 193–198.

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