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K Ikewaki, ... , L A Zech, H Nakamura

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Research Article

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Delayed Catabolism of High Density Lipoprotein Apolipoproteins A-I and A-II in Human Cholesteryl Ester Transfer Protein Deficiency

Katsunori Ikewaki,* Daniel J. Rader,* Takuya Sakamoto,* Masato Nishiwaki,[§] Naoki Wakimoto,^{||} Juergen R. Schaefer,* Toshitsugu Ishikawa,[§] Thomas Fairwell,* Loren A. Zech,* Haruo Nakamura,[§] Makoto Nagano,* and H. Bryan Brewer, Jr.*

*Molecular Disease Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892;

†Department of Internal Medicine, Aoto Hospital, Jikei University School of Medicine, Tokyo, Japan;

§First department of Internal Medicine, National Defense Medical College, Saitama, Japan;

and^{||}Department of Internal Medicine, Sapporo General Hospital, Hokkaido, Japan

Abstract

Deficiency of the cholesteryl ester transfer protein (CETP) in humans is characterized by markedly elevated plasma concentrations of HDL cholesterol and apoA-I. To assess the metabolism of HDL apolipoproteins in CETP deficiency, in vivo apolipoprotein kinetic studies were performed using endogenous and exogenous labeling techniques in two unrelated homozygotes with CETP deficiency, one heterozygote, and four control subjects. All study subjects were administered ¹³C₆-labeled phenylalanine by primed constant infusion for up to 16 h. The fractional synthetic rates (FSRs) of apoA-I in two homozygotes with CETP deficiency (0.135, 0.134/d) were found to be significantly lower than those in controls (0.196±0.041/d, *P* < 0.01). Delayed apoA-I catabolism was confirmed by an exogenous radiotracer study in one CETP-deficient homozygote, in whom the fractional catabolic rate of ¹²⁵I-apoA-I was 0.139/d (normal 0.216±0.018/d). The FSRs of apoA-II were also significantly lower in the homozygous CETP-deficient subjects (0.104, 0.112/d) than in the controls (0.170±0.023/d, *P* < 0.01). The production rates of apoA-I and apoA-II were normal in both homozygous CETP-deficient subjects. The turnover of apoA-I and apoA-II was substantially slower in both HDL₂ and HDL₃ in the CETP-deficient homozygotes than in controls. The kinetics of apoA-I and apoA-II in the CETP-deficient heterozygote were not different from those in controls. These data establish that homozygous CETP deficiency causes markedly delayed catabolism of apoA-I and apoA-II without affecting the production rates of these apolipoproteins. (*J. Clin. Invest.* 1993; 92:1650–1658.) Key words: cholesteryl ester transfer protein • kinetics • stable isotopes • high density lipoproteins • atherosclerosis

Introduction

Epidemiologic studies have consistently demonstrated that plasma concentrations of HDL cholesterol (HDL-C)¹ are in-

versely correlated with the incidence of coronary heart disease (CHD) (1, 2). Although the mechanisms by which HDL protects against atherosclerosis remain uncertain, HDL has been postulated to facilitate the efflux of cholesterol from peripheral tissues and transport it back to the liver in a process termed reverse cholesterol transport (3–5). The major protein constituents of HDL are apoA-I and apoA-II (6). Plasma concentrations of apoA-I are inversely correlated with CHD risk, whereas the association of apoA-II levels with CHD is not well established (7). Kinetic studies have established that plasma apoA-I levels are correlated with apoA-I catabolic rates, whereas plasma apoA-II levels are linked to apoA-II production rates in normolipidemic subjects (8–10). However, the factors regulating the metabolism of these apolipoproteins are not well understood.

Cholesteryl ester transfer protein (CETP) is a major factor modulating plasma HDL-C metabolism (11). CETP, a hydrophobic glycoprotein with a molecular weight of 74 kD, catalyzes the transfer of lipids among lipoproteins (11, 12). In humans, CETP-mediated transfer of cholesteryl ester (CE) from HDL to apoB-containing lipoproteins may be a major route of reverse cholesterol transport (13). The recent discovery that humans with CETP deficiency have markedly increased HDL cholesterol and apoA-I levels (14–17) supports the concept that CETP is an important factor regulating plasma HDL-C concentrations. However, the mechanism by which deficiency of CETP results in elevated apoA-I levels has not been established. To gain insight into the role of CETP in HDL apolipoprotein metabolism, we performed in vivo kinetic studies of apoA-I and apoA-II in two unrelated homozygotes with CETP deficiency, one heterozygote, and control subjects using both endogenous stable isotope and exogenous radiotracer techniques. The data establish that the catabolism of both apoA-I and apoA-II is significantly delayed in CETP deficiency.

Methods

Study subjects. Two unrelated homozygotes with CETP deficiency, one heterozygote, and four control subjects were investigated. The CETP-deficient subjects were referred to the National Defense Medical College, Saitama (subject 1) or Jikei University School of Medicine, Tokyo (subject 2) with markedly elevated plasma HDL-C levels. In both homozygous patients, plasma apoA-I levels were also increased, whereas apoA-II levels were in the upper range of normal. No CETP activity was detected in their plasma by a modification of the method by Tollefson and Albers (18). Sequencing of appropriate regions of the CETP gene after amplification by the polymerase chain reaction (19) established that both subjects were homozygous for the G to A substitution at the 5'-splice donor site of intron 14, which has been reported previously (17, 20, 21). A son of CETP-deficient subject 2, an obligate heterozygote, had an HDL-C of 61 mg/dl. The HDL₂-C levels in the CETP-deficient subjects were selectively increased, whereas HDL₃-C

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Address correspondence to Katsunori Ikewaki, M.D., Building 10, Room 7N117, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

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1. **Abbreviations used in this paper:** CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FCR, fractional catabolic rate; FSR, fractional synthetic rate; HDL-C, HDL cholesterol; PR, production rate; RT, residence time.

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levels were not different from controls. Consistent with previous reports (20, 22), the HDL₂-C/HDL₃-C ratios of the CETP-deficient homozygotes and the heterozygote were increased to 9.2, 12.3, and 3.0, respectively (normal 0.4±0.2). The molecular weights and isoelectric points of apoA-I and apoA-II were normal by SDS-PAGE and isoelectric focusing (23).

The clinical characteristics and lipid values of the study subjects are shown in Table I. Values are the mean of five fasting determinations made during the study. Plasma lipid and apolipoprotein concentrations remained in steady state throughout the study period. The four control subjects were healthy young females (mean age of 21 yr) and had normal fasting lipid and apolipoprotein levels. All study subjects had normal fasting plasma glucose levels and normal thyroid, hepatic, and renal function. The study protocol was approved by the Internal Review Boards of Jikei University School of Medicine and the National Heart, Lung and Blood Institute. Informed consent was given by each of the participants.

Endogenous stable isotope study protocol. After a 12-h fast, ¹³C₆-phenylalanine (99% ¹³C₆, Cambridge Isotope Laboratories, Woburn, MA) was administered as a priming bolus of 600 µg/kg, immediately followed by a constant infusion of 12 µg/kg per min for up to 12 to 16 h. Blood samples (20 ml) were obtained from the opposite arm at 10 min, 1, 2, 3, 4, 5, and 6 h, and every 2 h thereafter until the end of infusion. During the infusion meals were served in equal small portions every 2 h. Plasma was separated by centrifugation at 2,300 rpm for 30 min at 4°C.

Exogenous radiotracer study. ApoA-I was isolated from normal HDL (24) and stored at -20°C. Lyophilized apolipoprotein was solubilized in a buffer of 6 M guanidine-HCl and 1 M glycine (pH 8.5) and iodinated with ¹²⁵I by a modification of the iodine monochloride method, as previously reported (25). Iodination efficiency was 40% with incorporation of ~0.5 mole iodine per mole of protein. ¹²⁵I-apoA-I was reassociated with autologous lipoproteins and dialyzed extensively to remove free iodine. Human serum albumin was added to a final concentration of 5% (wt/vol). The injection samples were sterile-filtered through a 0.22-µm filter (Millipore Continental Water Systems, Bedford, MA) and tested for pyrogens and sterility. One day before the study, the homozygous CETP-deficient subject 1 was given potassium iodide (900 mg) in divided doses and this was continued throughout the study period. After a 12-h fast, 50 µCi of ¹²⁵I-apoA-I was injected into the CETP-deficient subject simultaneously with the stable isotope protocol above. Blood samples were obtained 10 min after the injection and then at selected time points through 14 d. Urine was collected continuously throughout the study. Sodium azide and apro-

tinin were added to the plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivity in plasma and urine was quantitated in a gamma counter (Cobra; Packard Instrument Co., Downers Grove, IL).

Isolation of lipoproteins. Total plasma lipoproteins were isolated from the plasma sample obtained at different time points after injection by ultracentrifugation at a density of 1.25 g/ml in a tabletop ultracentrifuge (TL-100; Beckman Instruments, Palo Alto, CA) using TLA-100.3 rotor at 100,000 rpm at 10°C for 10 h. Of total plasma 97.3% and 97.4% apoA-I and apoA-II were present in the top fraction. VLDL, IDL, LDL, HDL₂, and HDL₃ were isolated by sequential ultracentrifugation from 5 ml of plasma as previously described (26). In the homozygous CETP-deficient subjects, lipoproteins in the $d = 1.019$ – 1.063 g/ml range consisted of LDL as well as apoA-I-containing lipoproteins. These apoA-I-containing particles do not have apoB but are rich in apoE and have been referred to as HDL₁ (27, 28). To isolate the HDL₁ fraction, a polyclonal antiserum against apoB (Boehringer-Mannheim, Mannheim, Germany) was coupled to CNBr-Sepharose (5 mg/g gel) as reported previously (25) and lipoproteins of $d = 1.019$ – 1.063 g/ml from the homozygous CETP-deficient subjects were applied to this anti-apoB immunoaffinity column. The nonretained fraction (HDL₁) was collected and apolipoprotein composition was analyzed by SDS-PAGE.

Isolation of apolipoproteins. Lipoproteins were dialyzed against 10 mM ammonium bicarbonate, lyophilized, and delipidated. VLDL apoB-100 and total, HDL₁, HDL₂, and HDL₃ apoA-I were isolated by preparative gradient SDS-PAGE (5–15%) as previously reported (29). Total, HDL₂, and HDL₃ apoA-II were isolated by preparative isoelectric focusing (pH 4–6.5) (30). Apolipoproteins were identified by co-electrophoresis of purified apoA-I and apoA-II standards as well as by immunoblotting using monoclonal antibodies against apoB-100, apoA-I, and apoA-II.

Determination of isotopic enrichment. Samples were prepared for gas chromatography-mass spectrometry analysis as reported previously (29). Briefly, apolipoprotein bands were cut from gels, dried overnight (90°C), and subjected to hydrolysis in 6 N HCl (Ultrapure grade; J. T. Baker, Inc., Phillipsburg, NJ) at 110°C for 24 h. The protein hydrolysates were lyophilized in an evaporator (Speed-Vac; Savant Instrument, Inc., Farmingdale, NY). Plasma free amino acids and protein hydrolysates were purified by cation exchange chromatography (AG-50W-X8; Bio-Rad Laboratories, Richmond, CA). Recovered amino acids were derivatized to the *N*-heptafluorobutyl isobutyl esters, dissolved in ethyl acetate, and analyzed by gas chromatography-mass spectrometry (4500; Finnigan MAT, San Jose, CA) in the chemi-

Table I. Characterization of Study Subjects

Subjects	Sex	Age yr	BMI kg/m ²	TC	TG	HDL-C mg/dl	ApoA-I	ApoA-II
CETP deficiency								
Homozygotes								
1	M	42	26.0	251	98	177	210	37
2	F	67	18.7	282	79	170	216	39
Heterozygote*	M	40	24.2	145	67	61	126	29
Controls								
1	F	21	19.3	176	61	53	128	27
2	F	22	22.0	185	96	53	138	37
3	F	21	21.9	144	62	49	131	28
4	F	20	19.3	163	40	66	140	31
Mean		21	20.6	167	65	55	134	31
SD		0.7	1.3	15	20	6	5	4

* The CETP-deficient heterozygote is the son of the homozygous CETP-deficient subject 2. BMI; body mass index; TC, total cholesterol; TG, triglycerides.

cal ionization mode, using isobutane as the reagent gas. Selective ion monitoring at 418 m/z for unlabeled phenylalanine and 424 m/z for $^{13}\text{C}_6$ -phenylalanine was used to determine the isotope ratio. Each sample was analyzed at least three times. Enrichment was calculated from isotope ratio using the method by Cobelli et al. (31). The enrichment was then converted to the tracer/tracee ratio by using the formula: tracer/tracee ratio = $e(t)/[e_i - e(t)]$, where $e(t)$ is the enrichment of each sample at time t , and e_i is the enrichment of the infusate (31). In this study, e_i was 0.99.

Determination of kinetic parameters. For the endogenous labeling studies, the tracer/tracee ratio data of VLDL apoB-100, apoA-I, and apoA-II were fitted to monoexponential functions using SAAM 30 (32). The length of the constant infusion did not permit the use of the more than one exponential in the kinetic analysis of the data. The function was defined as: $A(t) = A_p(1 - e^{-(k(t-d))})$, where $A(t)$ is the tracer/tracee ratio at time t , A_p is the precursor pool tracer/tracee ratio for the apolipoprotein, k is the fractional synthetic rate (FSR), and d is the delay. The tracer/tracee ratios of VLDL apoB-100 and total apoA-I and apoA-II were simultaneously analyzed using the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool enrichment for VLDL apoB-100 and plasma apoA-I and apoA-II. We have found that apoA-I FSRs determined by monoexponential analysis of endogenous labeling data using the VLDL apoB-100 plateau tracer/tracee ratio as an estimate of the precursor pool tracer/tracee ratio for apoA-I synthesis were in excellent agreement (4% difference, $P = \text{NS}$) with those obtained simultaneously by the exogenous radiotracer method (Ikewaki, unpublished data). Apolipoprotein production rates (PR) were determined using the formula: $PR = (\text{FSR}) \times (\text{plasma apolipoprotein concentration}) \times (\text{plasma volume})/(\text{body weight})$ (33, 34). Plasma volume was assumed to be 4% of body weight.

For the exogenous radiotracer studies, plasma radioactivity curves were constructed by dividing the plasma radioactivity at each time point by the plasma radioactivity at the initial 10-min time point. The curves were fitted to biexponential functions using SAAM 30 (32). The length of the radiotracer study allowed the use of the biexponential in the kinetic analysis of the data. Residence times were obtained from the area under the curve. The fractional catabolic rate (FCR) is the reciprocal of the residence time. At steady state, the FCR is equal to the FSR. Despite the differences in data analysis between the two methods, the apoA-I kinetic parameters in one of the homozygous CETP-deficient patients were virtually identical.

Analytical methods. Total cholesterol and triglyceride levels were determined by automated enzymic techniques on an analyzer (VPSS; Abbott Laboratories, North Chicago, IL). HDL cholesterol was measured by dextran sulfate precipitation (35). Plasma apoA-I and apoA-II concentrations were quantitated using turbidimetric assays (Boehringer-Mannheim, Mannheim, Germany). ApoA-I concentrations in HDL₁ and LDL separated by anti-apoB immunoaffinity column were quantitated by ELISA as previously reported (36). Laser scanning densitometry (Ultrascan XI; LKB, Piscataway, NJ) of SDS-PAGE gels was used to determine the relative content of apoA-I and apoE in HDL₁.

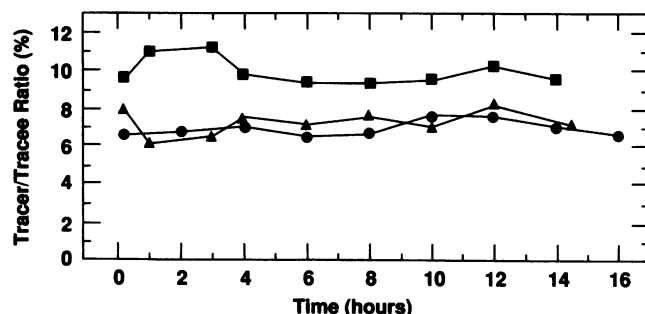


Figure 1. Tracer/tracee ratios of free plasma phenylalanine in homozygous CETP-deficient subject 1 (circles) and subject 2 (squares) and a heterozygous CETP-deficient subject (triangles).

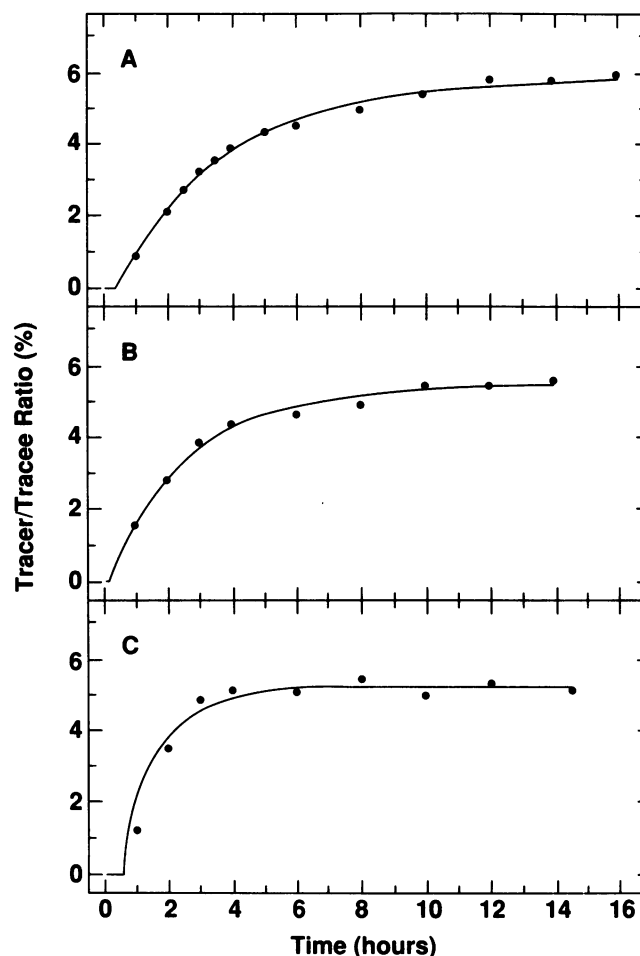


Figure 2. Tracer/tracee ratios of VLDL apoB-100 in homozygous CETP-deficient subject 1 (A) and subject 2 (B) and a heterozygous CETP-deficient subject (C). Data were fitted by monoexponential function analysis as described in the methods section.

Results

The tracer/tracee ratio curves of free plasma phenylalanine during the constant infusion in the two CETP-deficient homozygotes and the heterozygote are illustrated in Fig. 1. In all study subjects, the tracer/tracee ratio of free plasma phenylalanine increased rapidly after the priming bolus, then remained relatively constant throughout the infusion period. The mean tracer/tracee ratios of free plasma phenylalanine were 6.8 and 9.8% in the homozygous CETP-deficient subjects 1 and 2, respectively, and 7.2% in the heterozygous subject. The mean tracer/tracee ratio of free plasma phenylalanine in the four control subjects was $5.6 \pm 0.2\%$.

The tracer/tracee ratios of VLDL apoB-100 during the constant infusion in the CETP deficient subjects are shown in Fig. 2. Monoexponential function analysis determined the VLDL apoB-100 plateau tracer/tracee ratios to be 5.9% in the homozygous CETP-deficient subject 1, 5.4% in subject 2, and 5.2% in the heterozygous subject. The mean VLDL apoB-100 plateau tracer/tracee ratio in the four controls was $5.2 \pm 0.5\%$.

The apoA-I tracer/tracee ratio curves are illustrated in Fig. 3. In both homozygous CETP-deficient subjects, the apoA-I tracer/tracee ratio curves had shallower slopes than did the

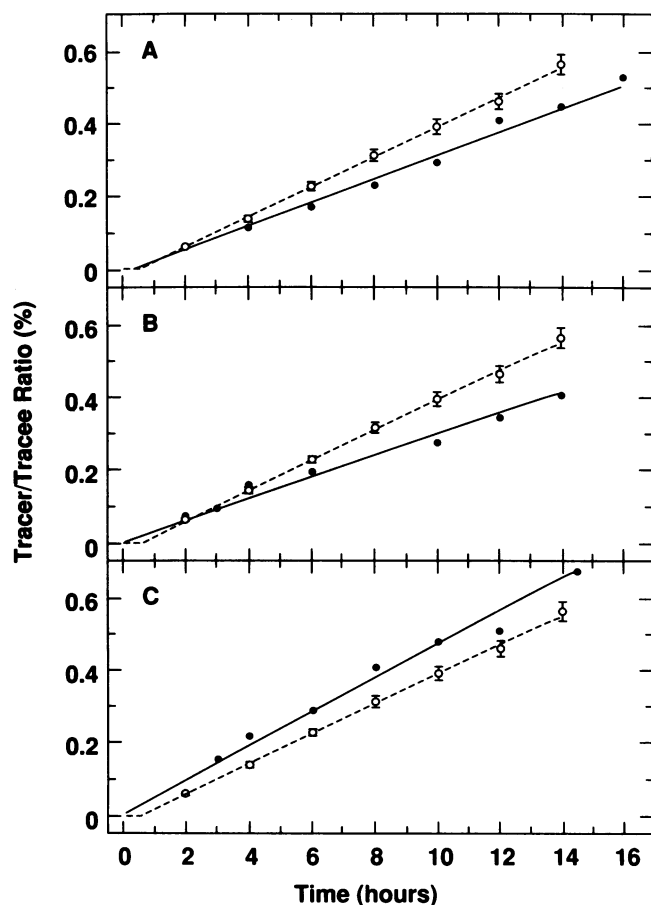


Figure 3. Tracer/tracee ratios of total plasma apoA-I (solid circles) in homozygous CETP-deficient subject 1 (A) and subject 2 (B) and a heterozygous CETP-deficient subject (C) with the mean tracer/tracee ratio of control subjects (open circles with dotted line). Data from control subjects are given as the mean \pm SD. Data were fitted by mono-exponential function analysis with the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-I.

curve of the mean of four control subjects, indicating a delayed apoA-I catabolism. However, the apoA-I tracer/tracee ratio curve in the heterozygous subject was similar to the mean of

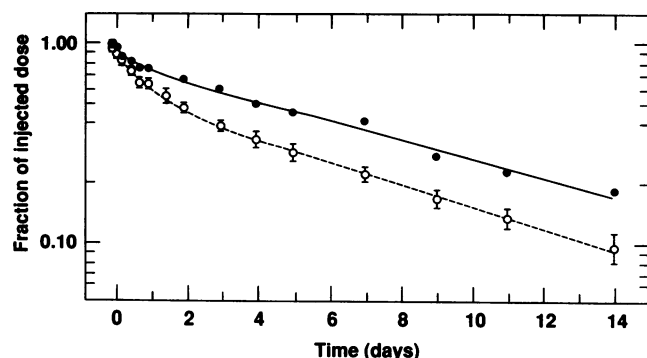


Figure 4. Metabolism of ^{125}I -apoA-I in homozygous CETP-deficient subject 1 (solid circles) and the mean of 10 control subjects (open circles with dotted line). Data from control subjects are given as the mean \pm SD.

control subjects. The ^{125}I -apoA-I plasma curve in homozygous CETP-deficient subject 1 is shown in Fig. 4 with the mean ^{125}I -apoA-I plasma curve of 10 normal control subjects. The exogenous apoA-I radiotracer study confirmed the delayed apoA-I catabolism in this CETP-deficient subject. The apoA-II tracer/tracee ratio curves are illustrated in Fig. 5. They also demonstrate shallower slopes in the CETP-deficient homozygotes, consistent with slower turnover of apoA-II in these subjects.

The kinetic parameters of total plasma apoA-I and apoA-II are summarized in Table II. In the two CETP-deficient homozygotes, the mean FSR of total plasma apoA-I was decreased by 31% compared with that in control subjects ($P < 0.01$). The apoA-I FCR obtained by the exogenous radiotracer method (0.139/d) was highly comparable with that obtained by endogenous labeling (0.135/d) in the homozygous CETP-deficient subject 1. The mean FSR of apoA-II was decreased by 36% in the CETP-deficient homozygotes compared with that in the controls ($P < 0.01$). As in the control subjects, the apoA-II FSRs were lower than the apoA-I FSRs in both CETP-deficient

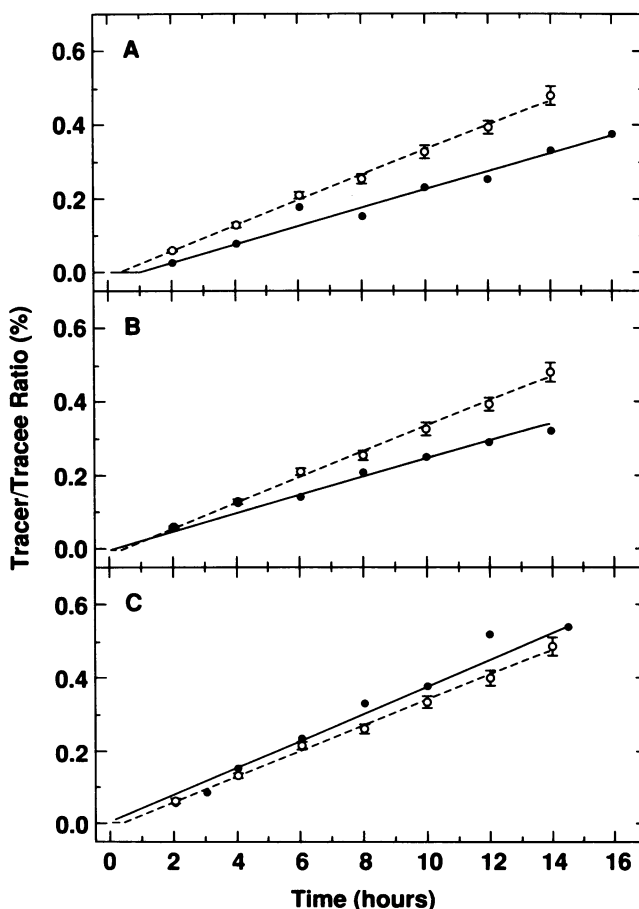


Figure 5. Tracer/tracee ratios of total plasma apoA-II (solid circles) in homozygous CETP-deficient subject 1 (A) and subject 2 (B) and a heterozygous CETP-deficient subject (C) with the mean tracer/tracee ratio of control subjects (open circles with dotted line). Data from control subjects are given as the mean \pm SD. Data were fitted by mono-exponential function analysis with the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-II.

Table II. Kinetic Parameters of Total Plasma apoA-I and apoA-II in Subjects with CETP Deficiency

Subjects	ApoA-I			ApoA-II		
	Conc.	FSR	PR	Conc.	FSR	PR
	mg/dl	d ⁻¹	mg/kg-d	mg/dl	d ⁻¹	mg/kg-d
CETP deficiency						
Homozygotes						
1	210	0.135±0.022 (0.139±0.002)*	11.4±1.8	37	0.104±0.017	1.55±0.25
2	216	0.134±0.020	11.6±1.7	39	0.112±0.017	1.74±0.26
Heterozygote	126	0.229±0.016	11.5±0.8	29	0.182±0.022	2.11±0.26
Controls						
1	138	0.217±0.031	12.0±1.7	36	0.210±0.030	3.05±0.43
2	128	0.191±0.025	9.8±1.3	27	0.153±0.016	1.64±0.17
3	140	0.174±0.062	9.7±3.5	31	0.160±0.053	1.99±0.66
4	131	0.201±0.046	10.5±2.4	28	0.156±0.027	1.72±0.30
Mean±SD	134±5	0.196±0.041†	10.5±0.9	31±4	0.170±0.023‡	2.10±0.39

FSRs were determined using the monoexponential functions with the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-I and apoA-II. Values for kinetic parameters are given as the best estimate±SD. FSR, fractional synthetic rate; PR, production rate. * Value obtained by ¹²⁵I-apoA-I radiotracer study. † *P* < 0.01 compared with the mean of the CETP-deficient homozygotes.

homozygotes, demonstrating that apoA-II has a slower turnover than apoA-I in CETP-deficient as well as in normal subjects. The production rates of apoA-I were within the normal range in both homozygous CETP-deficient subjects. The mean apoA-II production rate in the CETP-deficient homozygotes was 22% less than that of controls, but was not significantly different (*P* = 0.27). The heterozygous subject had normal apoA-I and apoA-II FSRs and production rates.

The apolipoprotein composition of HDL₁ separated by an anti-apoB immunoaffinity column from the *d* = 1.019–1.063 g/ml lipoproteins of homozygous CETP-deficient subject 2 is illustrated in Fig. 6. The HDL₁ was enriched in apoE (apoE/apoA-I mass ratio = 0.50 in the CETP-deficient homozygote 1 and 0.82 in the homozygote 2, compared with 0.02 in controls). ApoA-I was not detectable in LDL (the retained fraction) by ELISA. The tracer/tracee ratio curves of apoA-I from this apoE-rich HDL₁ fraction as well as from HDL₂ and HDL₃ are shown in Fig. 7, and the kinetic parameters of apoA-I and apoA-II in these HDL subfractions are shown in Table III. Although apoA-I HDL₁ FSRs were somewhat increased compared with apoA-I HDL₂ FSRs, the difference was not statisti-

cally significant (*P* = 0.10). Both the HDL₂ and HDL₃ apoA-I and apoA-II FSRs were significantly slower in both CETP-deficient homozygotes compared with corresponding FSRs in control subjects, whereas those in the CETP-deficient heterozygote were not different from control subjects.

Discussion

Although plasma HDL-C levels are highly correlated with plasma apoA-I levels (37), the catabolic rate of HDL-C in humans is much faster than that of apoA-I (38), indicating a dissociation of HDL-C and HDL apolipoprotein metabolism. CETP deficiency in humans results in markedly elevated plasma HDL-C levels, presumably due to the inability to transfer CE from HDL into apoB-containing lipoproteins. However, plasma concentrations of apoA-I are increased in CETP deficiency as well (14, 22). The major goal of the present study was to determine the effect of the lack of CETP on the *in vivo* metabolism of HDL apolipoproteins A-I and A-II.

We used both endogenous stable isotope and exogenous radiotracer labeling techniques to approach the problem. The use of a primed constant infusion of a stable isotopically labeled amino acid to endogenously label apolipoproteins has been reported by several investigators (29, 39–47). This method requires the ability to reliably estimate the tracer/tracee ratio of the precursor pool for apolipoprotein synthesis. The plateau tracer/tracee ratio of VLDL apoB-100, a primarily liver-derived protein, has been used as an estimate for the apoA-I precursor tracer/tracee ratio (40, 41). However, because apoA-I is known to be synthesized in the small intestine as well as the liver (48, 49), and since evidence suggests that the precursor pool tracer/tracee ratio may not be the same in these two organs (45, 50), theoretical concerns have been raised that the VLDL apoB-100 plateau may not serve as a reliable estimate for apoA-I precursor tracer/tracee ratio. We have investi-

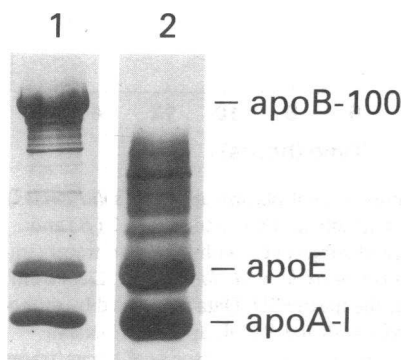


Figure 6. SDS-PAGE of apolipoproteins in lipoproteins from homozygous CETP-deficient subject 2. Lipoproteins (*d* = 1.019–1.063 g/ml, lane 1) were applied to an anti-apoB-100 immunoaffinity column and the non-retained fraction (HDL₁, lane 2) was isolated.

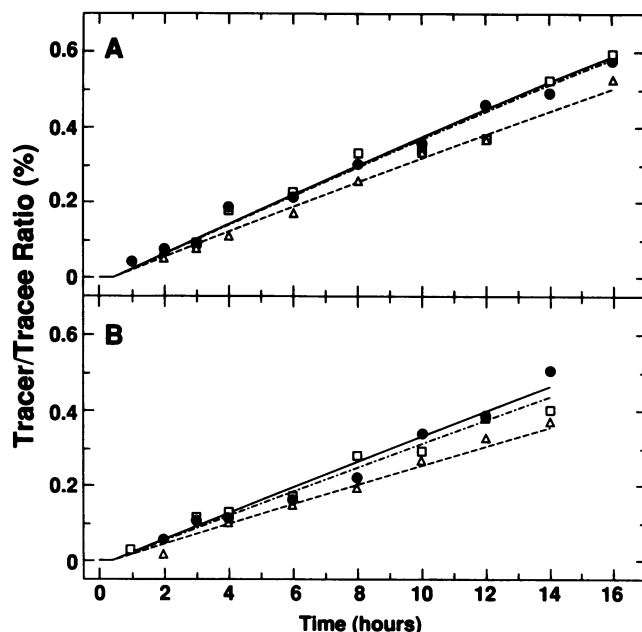


Figure 7. Tracer/tracee ratios of apoA-I in HDL₁ (solid circles with solid line), HDL₂ (open triangles with dotted line), and HDL₃ (open squares with dashed line) in homozygous CETP-deficient subject 1 (A) and subject 2 (B). Data were fitted by monoexponential function analysis with the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-I.

gated apoA-I kinetics by simultaneous endogenous and exogenous labeling in several subjects with a wide range of plasma apoA-I levels and have found that the use of the VLDL apoB-100 plateau tracer/tracee ratio resulted in apoA-I kinetic parameters by endogenous labeling that were highly comparable (only 4% difference) with those obtained by exogenous radio-tracer techniques (Ikewaki, unpublished data). Similarly, in

the current study, the apoA-I turnover rates determined by simultaneous endogenous and exogenous labeling in homozygous CETP deficient subject 1 were virtually identical. Moreover, in a primed constant infusion stable isotope study in a patient with extremely low apoA-I, the apoA-I reached a plateau tracer/tracee ratio during the infusion that was 91% of the VLDL apoB-100 plateau tracer/tracee ratio in the same subject (50a). These combined data suggest that the use of the VLDL apoB-100 plateau tracer/tracee ratio as an estimate of the precursor pool tracer/tracee ratio for apoA-I synthesis is a reasonable approach for the determination of apoA-I turnover using primed constant infusion endogenous labeling.

Both CETP-deficient homozygotes were found to have significantly decreased rates of catabolism of apoA-I with normal production rates, indicating that the elevated apoA-I levels were due solely to the delayed apoA-I catabolism. The plasma apoA-II levels were not elevated to the same degree as those of apoA-I, consistent with findings by other investigators (14, 16, 22). However, the CETP-deficient homozygotes also had substantially delayed apoA-II catabolism; the apoA-II production rates were somewhat lower than the controls, but the difference was not statistically significant. Thus, both apoA-I and apoA-II catabolism is delayed in CETP deficiency. It is possible that a secondary decrease in the production rate of apoA-II might partially offset the effect of delayed catabolism, resulting in less marked elevations in plasma apoA-II concentrations.

Our control subjects were somewhat younger than the CETP-deficient subjects. However, in a study by Fidge et al. (51), there was no significant correlation between age and apoA-I FCR ($r = 0.043$, $P = 0.86$), whereas apoA-I and HDL-C levels were inversely correlated with apoA-I FCR's. Given that fact, together with data reported in the Lipid Research Clinic prevalence study (52) and by Stevenson et al. (53) in which HDL-C levels were not different in different age groups, it is highly unlikely that the use of younger controls affected our conclusions. In addition, Hazzard et al. (54) reported that the catabolism of apoA-I in women was delayed by the treatment

Table III. FSRs of apoA-I and apoA-II of HDL₁, HDL₂, and HDL₃ in Subjects with CETP Deficiency

Subjects	ApoA-I			ApoA-II	
	HDL ₁	HDL ₂	HDL ₃	HDL ₂	HDL ₃
d^{-1}					
CETP deficiency					
Homozygotes					
1	0.161±0.007	0.137±0.006	0.160±0.006	0.100±0.009	0.104±0.004
2	0.158±0.009	0.114±0.006	0.137±0.007	0.096±0.005	0.121±0.007
Heterozygote		0.228±0.009	0.240±0.011	0.139±0.007	0.185±0.009
Controls					
1		0.242±0.018	0.270±0.013	0.176±0.011	0.202±0.011
2		0.207±0.007	0.208±0.006	0.145±0.007	0.147±0.006
3		0.199±0.005	0.216±0.005	0.150±0.004	0.160±0.004
4		0.213±0.008	0.220±0.008	0.155±0.007	0.166±0.007
Mean±SD		0.215±0.009 [‡]	0.229±0.008*	0.156±0.007 [‡]	0.169±0.007*

FSRs were determined using the monoexponential functions with the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-I and apoA-II. Values for FSRs are given as the best estimate±SD. * $P < 0.05$, [‡] $P < 0.01$ compared with the mean of the CETP deficient homozygotes.

with estrogen, which suggests that it is unlikely that the estrogen-deficient postmenopausal status of one of our homozygous subjects (CETP-deficient subject 2) accounted for her delayed apoA-I catabolism.

Our current observations establish that CETP deficiency causes elevated plasma apoA-I and apoA-II levels primarily by modulating their catabolism. However, Brinton et al. (9) reported that female subjects with comparably high plasma apoA-I levels but presumably normal CETP activities had also delayed apoA-I catabolism. Therefore, factors other than CETP deficiency can result in delayed apoA-I catabolism. In addition, we have reported a case with a similarly elevated apoA-I level due to increased apoA-I production (55). Thus, it remains to be determined whether variation in CETP activity plays an important role in regulating plasma apoA-I levels in normal or dyslipidemic populations.

The mechanism of the delayed apoA-I and apoA-II catabolism in humans with CETP deficiency may be related to increased HDL core lipid and particle size. Data from our laboratory (25) and others (9, 56, 57) suggest that HDL particle size may affect metabolism, with smaller particles having a faster catabolism. Since HDL particles in CETP-deficient homozygotes are extremely enlarged and lipid enriched, the conformation of apoA-I and apoA-II may be altered, resulting in the decreased cellular uptake and catabolism (58). CETP is believed to participate in the conversion of larger HDL₂ to smaller HDL₃ (5, 59, 60). During this process, some HDL apolipoproteins may be transferred to triglyceride-rich lipoproteins (11). Both the conversion to smaller HDL particles and transfer of apolipoproteins to triglyceride-rich lipoproteins may result in faster apolipoprotein catabolism. Since this process is interrupted in CETP deficiency, the relative proportion of apoA-I and apoA-II catabolized by these relatively rapid pathways may be decreased, thus delaying the overall catabolic rates of these apolipoproteins.

Despite the fact that HDL₂ is selectively increased in CETP deficiency, the turnover of apoA-I and apoA-II was found to be decreased in both HDL₂ and HDL₃ in the CETP-deficient homozygotes, indicating that lack of CETP affects HDL apolipoprotein metabolism throughout the entire HDL density spectrum. Furthermore, although the difference was not statistically significant, the catabolism of apoA-I in HDL₁ was somewhat faster than that in HDL₂ in both CETP-deficient homozygotes, possibly due to the enrichment of apoE on the HDL₁ particles. This in vivo evidence is consistent with the in vitro observation that HDL₁ from a CETP-deficient subject had greater affinity for the fibroblast LDL receptor than HDL from a normal subject (27). Furthermore, a metabolic study in cholesterol-fed baboons demonstrated that HDL₁ was catabolized more rapidly than normal HDL (61). Although speculative, these results suggest that apoE-mediated catabolism of HDL may be one of the pathways for HDL apolipoprotein catabolism (and possibly reverse cholesterol transport) in CETP deficiency. However, the catabolic rates of HDL₁ apoA-I in the CETP-deficient homozygotes are still substantially slower than that of normal HDL apoA-I in control subjects. Furthermore, apoE-rich HDL accumulate in the plasma of CETP-deficient subjects, indicating that the effect of the apoE is not enough to fully compensate for the delayed apoA-I catabolism caused by the CETP deficiency.

Although cholesterol distribution in HDL has been shown

to be altered in heterozygous CETP deficiency, plasma apoA-I and apoA-II levels vary from normal to slightly higher than normal (20, 22). In this study, the heterozygous CETP-deficient subject had normal apoA-I and apoA-II levels and no significant differences in apoA-I and apoA-II metabolism compared with the controls. However, further studies will be required to determine whether heterozygosity for CETP deficiency has an effect on HDL apolipoprotein metabolism or only on HDL-C metabolism.

It has been suggested, but remains to be proven, that the high levels of HDL-C and apoA-I in CETP deficiency may be protective against the development of atherosclerosis (14, 20). Recent studies in transgenic mice overexpressing human apoA-I (62) suggest a protective role of increased apoA-I biosynthesis. The present study demonstrates that the elevated levels of apoA-I in CETP deficiency are due solely to delayed catabolism, which may not have the same physiologic effect on reverse cholesterol transport or atherogenesis as increased apoA-I biosynthesis. The elevated HDL-C and apoA-I could exert a protective effect by a mechanism distinct from reverse cholesterol transport, perhaps by preventing the formation of oxidized LDL (63, 64). The low plasma LDL cholesterol and apoB levels in CETP-deficient subjects (20) may also play a protective role. Therefore, both increased HDL-C and decreased LDL-C levels could potentially contribute to the decreased risk of atherosclerosis possibly associated with CETP deficiency.

In summary, complete deficiency of CETP results in substantially delayed catabolism of apoA-I and apoA-II with normal production rates of these apolipoproteins. ApoA-I and apoA-II kinetics were normal in a heterozygous CETP-deficient subject. This study provides new insights into the role of CETP in modulating HDL apolipoprotein metabolism and possibly the risk of premature cardiovascular disease.

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