Plasma turnover of HDL apoC-I, apoC-III, and apoE in humans: in vivo evidence for a link between HDL apoC-III and apoA-I metabolism

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Abstract **Numerous factors are known to affect the plasma metabolism of HDL, including lipoprotein receptors, lipid transfer protein, lipolytic enzymes and HDL apolipoproteins. In order to better define the role of HDL apolipoproteins in determining plasma HDL concentrations, the aims of the present study were: *a*) to compare the in vivo rate of plasma turnover of HDL apolipoproteins [i.e., apolipoprotein A-I (apoA-I), apoC-I, apoC-III, and apoE], and *b*) to investigate to what extent these metabolic parameters are related to plasma HDL levels. We thus studied 16 individuals with HDL cholesterol levels ranging from 0.56–1.66 mmol/l and HDL apoA-I levels ranging from 89–149 mg/dl. Plasma kinetics of HDL apolipoproteins were investigated using a primed constant (12 h) infusion of deuterated leucine. Plasma HDL apolipoprotein levels were 41.8 ± 1.5, 9.7 ± 0.5, 4.9 ± 0.5, and 0.7 ± 0.1 μmol/l for apoA-I, apoC-I, apoC-III and apoE. Plasma transport rates (TRs) were 388.6 ± 24.7, 131.5 ± 12.5, 66.5 ± 9.1, and 31.4 ± 3.3 nmol·kg^{-1}·day^{-1}; and residence times (RTs) were 5.1 ± 0.4, 3.7 ± 0.3, 3.6 ± 0.3, and 1.1 ± 0.1 days, respectively. HDL cholesterol and apoA-I levels were significantly correlated with HDL apoA-I RT ($r = 0.69$ and $r = 0.56$), and were not significantly correlated with HDL apoA-I TR. In contrast, HDL apoC-I, apoC-III, and apoB levels were all positively related to their TRs and not their RTs. HDL apoC-III TR was postively correlated with levels of HDL apoC-III ($r = 0.73$, $P < 0.01$), and with those of HDL cholesterol and apoA-I ($r = 0.54$ and $r = 0.53$, $P < 0.05$, respectively). HDL apoC-III TR was in turn related to HDL apoA-I RT ($r = 0.51$, $P < 0.05$). Together, these results provide in vivo evidence for a link between the metabolism of HDL apoC-III and apoA-I, and suggest a role for apoC-III in the regulation of plasma HDL levels.**—Cohn, J. S., R. Batal, M. Tremblay, H. Jacques, L. Veilleux, C. Rodriguez, O. Mamer, and J. Davignon. **Plasma turnover of HDL apoC-I, apoC-III, and apoE in humans: in vivo evidence for a link between HDL apoC-III and apoA-I metabolism. *J. Lipid Res.* 2003.** 44: **1976–1983.**

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Low plasma levels of HDL are associated with increased risk of coronary heart disease (CHD) (1, 2). A strong therapeutic rationale therefore exists for increasing plasma HDL levels of patients at risk for CHD. An ongoing search for novel therapeutic agents able to increase plasma HDL levels (3) depends on a clear understanding of the many metabolic factors that affect plasma HDL metabolism. These include *a*) lipoprotein receptors, e.g., scavenger receptor class B type I (SR-BI) and the adenosine triphosphate binding cassette transporter (ABCA1) (4, 5); *b*) lipid transfer proteins, e.g., cholesteryl ester transfer protein and phospholipid transfer protein (6); *c*) lipolytic enzymes, e.g., lipoprotein lipase, hepatic lipase (HL), and endothelial lipase (7); and *d*) HDL apolipoproteins, e.g., apolipoprotein A-I (apoA-I) and apoA-II (8).

Many studies have investigated the physiological role of apolipoproteins that readily exchange between plasma lipoproteins (i.e., apoC-I, apoC-III, and apoE). The majority of this work has focused on the effect of these proteins on triglyceride-rich lipoprotein (TRL) metabolism; however, there is ample evidence that these proteins can also directly affect plasma HDL metabolism (9, 10). For example, apoC-I has been shown to activate LCAT (11–13), inhibit HL (14, 15), inhibit phospholipase A_2 (16), and also reduce cholesterol ester transfer protein activity (17, 18). ApoC-III and apoE have both been shown to interact with SR-BI (19, 20) and ABCA1 (21). ApoE can also reduce HL activity (22) and inhibit cubilin-mediated HDL endocytosis (23). Direct effects of apoC-I, apoC-III, and apoE on HDL metabolism are in addition to indirect effects, whereby stimulation or inhibition of TRL catabolism by these proteins can subsequently cause a reciprocal change in HDL formation.

Abbreviations: CHD, coronary heart disease; HL, hepatic lipase; SR-BI, scavenger receptor class B type I; TR, transport rate; TRL, triglyceride-rich lipoprotein.

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In order to better define the role of HDL apolipoproteins in determining plasma HDL concentration, the aims of the present study were *a*) to compare the in vivo rate of plasma turnover of HDL apolipoproteins (i.e., apoA-I, apoC-I, apoC-III, and apoE), and *b*) to investigate to what extent these metabolic parameters are related to plasma HDL levels. Data were obtained from 16 individuals who had previously participated in stable isotope infusion studies focusing on the metabolism of apoC-I, apoC-III, and/or apoE in TRL (24–26). These subjects had quite different HDL concentrations, with a 3-fold range in HDL cholesterol and a 2-fold range in plasma apoA-I. Because the intravenous infusion of deuterated leucine in a stable isotope study results in the incorporation of labeled leucine into all plasma proteins, we were able to retrospectively isolate apoA-I from stored blood samples taken from study subjects and directly measure apoA-I kinetic parameters for these individuals. This provided a unique opportunity to compare plasma mass and kinetic parameters for apoE, apoC-I, apoC-III, and apoA-I in HDL.

METHODS

Study subjects

Plasma apolipoprotein samples were analyzed from 16 individuals (15 males and one female) who had participated in previous stable-isotope kinetic studies (24–26). They were apparently healthy individuals, aged from 25 to 67 years, with a body mass index (BMI) from 23.5 to 30.2 kg/m^2 (**Table 1**) having normal or elevated plasma lipid levels. They were not taking medications known to affect plasma lipid levels. They had a mean (±SE) plasma triglyceride concentration of 2.60 ± 0.44 mmol/l, a mean plasma cholesterol concentration of 5.68 ± 0.43 mmol/l, and an LDL cholesterol concentration of 3.68 ± 0.40 mmol/l. Their plasma HDL cholesterol concentrations ranged from 0.56 to 1.66 mmol/l, and their plasma apoA-I ranged from 89 to 149 mg/dl (Table 1). All subjects gave informed consent to the study protocol, which was approved by the Ethics Committee of the Clinical Research Institute of Montreal.

TABLE 1. Subject characteristics

Subject	Age	BMI	Plasma Triglyceride	Plasma Cholesterol	LDL Cholesterol	HDL Cholesterol	ApoA-I
	years	*kg/m^2*		*mmol/l*			*mg/dl*
1	25	30.2	2.06	7.53	6.06	0.71	89
2	26	25.9	0.80	3.55	2.36	0.98	101
3	40	29.3	3.14	3.57	1.81	0.78	102
4	35	26.1	1.56	4.75	3.38	0.94	105
5	63	25.5	2.36	8.34	6.28	1.33	107
6	38	24.7	5.87	6.15	3.02	0.59	111
7	59	28.2	4.17	7.18	3.91	1.28	112
8	58	26.8	3.19	6.81	4.33	1.20	116
9	40	24.4	1.42	8.02	6.77	0.78	119
10	67	24.1	6.25	6.46	2.38	0.56	121
11	26	24.4	0.72	3.18	1.88	1.19	122
12	41	24.1	2.22	5.79	4.09	1.03	129
13	63	26.6	4.30	4.13	2.69	0.56	130
14	38	25.4	2.16	6.86	4.73	1.20	134
15	30	23.5	0.69	3.76	2.19	1.57	147
16	36	24.5	0.67	4.78	2.92	1.66	149

ApoA-I, apolipoprotein A-I; BMI, body mass index. Plasma lipid and apolipoprotein concentrations for each fasted subject represent the average of measurements for five blood samples taken at 3 h intervals during the stable-isotope infusion experiment. Subjects are presented in order of increasing plasma apoA-I concentration.

Stable isotope infusion

After a 12 h overnight fast, subjects were given a primed constant intravenous infusion of deuterium-labeled leucine (L-[D_3]leucine 98%, Cambridge Isotope Laboratories, MA), as previously described (27, 28). They were injected via a needle attached to a left forearm vein with 10 μmol per kg body weight of L-[D_3]leucine, dissolved in physiological saline, followed by a 12 h constant infusion (given by peristaltic pump) of 10 μmol L-[D_3]leucine/kg/h. Subjects remained fasted during the infusion but had free access to drinking water. Blood samples (20 ml) were collected from an antecubital vein of the right arm at regular intervals (0, 15, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 h) in tubes containing EDTA to a final concentration of 0.1%. Plasma was immediately separated by centrifugation at 3,500 rpm for 15 min at 4°C. An antimicrobial agent (sodium azide) and a protease inhibitor (aprotinin) were added to plasma samples to give a final concentration of 0.02% and 1.67 μg/ml, respectively.

Lipids, lipoproteins, and apolipoproteins

VLDL, IDL+LDL, HDL, and a d > 1.21 g/ml fraction were isolated from 5 ml of plasma by sequential ultracentrifugation in an XL-90 ultracentrifuge using a 50.4 Ti rotor (Beckman, 50,000 rpm for 10 h), at densities (d) of 1.006 g/ml, 1.063 g/ml, and 1.21 g/ml, respectively. All lipoprotein fractions were recovered by tube slicing and were adjusted to a volume of 2.5 ml with physiological saline. ApoC-I in HDL was separated from other apolipoproteins by preparative electrophoresis using polyacrylamide-urea (8 M) gels (pH 9.1) (29). ApoA-I, apoC-III, and apoE were separated by preparative isoelectric focusing on 7.5% polyacrylamide-urea (8 M) gels (pH gradient 4–7) (30). Coomassie blue staining was used to identify the position of apolipoproteins in gels after electrophoresis. Plasma and lipoprotein fractions were assayed for total (free and esterified) cholesterol and triglyceride with a COBAS MIRA-S automated analyzer (Hoffmann-LaRoche) using enzymatic reagents. Plasma apoA-I concentrations were measured by nephelometry on a Behring Nephelometer 100 (Behring) using Behring protocol and reagents. Because >90% of plasma apoA-I was consistently found in the HDL fraction after ultracentrifugation (1.063 < d < 1.21 g/ml), plasma apoA-I concentrations were taken to represent HDL apoA-I levels. Plasma and HDL apoC-I, apoC-III, and apoE concentrations were measured with ELISAs developed in our laboratory (26, 31, 32). Immunopurified polyclonal goat anti-human antibodies (Biodesign, Kennebunk, ME) were used for both capture and detection. Assays were calibrated with standard plasmas kindly provided by Dr. Petar Alaupovic (Oklahoma Medical Research Foundation, Oklahoma City). Intraassay and interassay coefficients of variation were between 2% and 10%. As explained before (24–26), apoC-I, apoC-III, and apoE recovered in the d > 1.21 g/ml fraction (1.9 ± 0.4%, 0.5 ± 0.1%, and 14.6 ± 0.9% of total, respectively) were included in HDL fractions, which were in turn corrected so that total apolipoprotein recovery after ultracentrifugation was 100%.

Determination of isotopic enrichment

Apolipoprotein bands, as well as blank (nonprotein containing) gel slices were excised from polyacrylamide gels. Each slice was added to a borosilicate sample vial containing 600 μl of 6 N HCL, and an internal standard of 250 ng norleucine (Sigma Aldrich) dissolved in 50 μl double-distilled water. Gel slices were hydrolyzed at 110°C for 24 h, cooled to −20°C for 20 min, and

centrifuged at 3,500 rpm for 5 min. Free amino acids in the hydrolysate were separated from precipitated polyacrylamide, purified by cation exchange chromatography using AG 50 W-X8 resin (BioRad), and derivatized by treatment with 200 μl of acetyl chloride-acidified 1-propanol (1:5; v/v) for 1 h at 100°C, and 50 μl of heptaflurobutyric anhydride (Supelco) for 20 min at 60°C (27). Plasma amino acids were also separated by cation exchange chromatography and derivatized to allow for the determination of plasma leucine isotopic enrichment. Enrichment of samples with deuterium-labeled leucine was measured by gas chromatography-mass spectrometry (Hewlett-Packard, 5988) using negative chemical ionization and methane as the moderator gas. Selective ion monitoring at $m/z = 352$ and 349 (ionic species corresponding to derivatized deuterium-labeled and derivatized nondeuterium-labeled leucine, respectively) was performed, and tracer to tracee ratios (%ttr) were derived from isotopic ratios for each sample. %Ttrs were corrected for background leucine in gel slices (and for leucine introduced during the amino acid purification and derivitization procedures) by estimating the amount of leucine in processed blank gel slices in relation to the norleucine internal standard. Background leucine represented 10% or less of total leucine recovered in apolipoprotein samples.

Kinetic analysis

Stable isotope enrichment curves for HDL apolipoproteins were fitted to a three-compartment model, as carried out previously (24–26) using SAAM II computer software (SAAM II Institute, Seattle, WA). The first compartment represented the plasma amino acid precursor pool. The second compartment was a delay compartment, which accounted for the synthesis, assembly, and secretion of apolipoprotein. The third compartment was the plasma protein compartment. Plasma leucine enrichment (measured eight times during the course of the infusion experiment) was used as a measure of precursor pool enrichment. VLDL apoB-100 enrichment at plateau is a better estimate of precursor pool enrichment, but inasmuch as this plateau was not well defined in some subjects with elevated VLDL apoB-100, plasma leucine was used for all individuals. (Because the plasma leucine plateau is usually 10% to 15% higher than that of VLDL apoB-100, this may have resulted in a 10–15% underestimation of FCRs for all apolipoproteins). Mean (±SE) plasma leucine %ttr was 10.42 ± 0.56% (n = 16). Modeling of %ttr data resulted in the determination of fractional transport rates (FTRs) (i.e., the fraction of HDL apolipoprotein pools being renewed per day). Residence time (RT) was calculated as the reciprocal of FTR (1/FTR), and transport rate (TR) was calculated (in $mg \cdot kg^{-1} \cdot day^{-1}$) as in Equation 1:

$$TR = FTR\,(pools/day) \times \frac{\text{apolipoprotein pool size (mg)}}{\text{body weight (kg)}} \qquad \textit{(Eq. 1)}$$

where pool size = plasma concentration (mg/dl) × plasma volume (0.045 l/kg).

Plasma apolipoprotein levels and HDL apolipoprotein TRs were expressed in molar units (μmol/l and nmol/kg·day) using a molecular weight of 6,613 Da for apoC-I, 8,746 Da for apoC-III, 34,200 Da for apoE, and 28,331 Da for apoA-I.

Statistical analysis

Statistical significance of differences between mean values was assessed by paired Student's *t*-tests using SigmaStat software (Jandel Scientific, San Rafael, CA). Student's *t*-test results were adjusted for multiple comparisons with a Bonferroni correction. Pearson correlation coefficients (r) were calculated to describe the correlation between different kinetic and mass parameters.

RESULTS

Characteristics of study subjects

Plasma lipid and apolipoprotein concentrations of study subjects (n = 16) in the fasted state on the day of the stable-isotope infusion experiment are shown in Table 1. Subject 13 was a female. Each value represents the average of five measurements taken at 3 h intervals during the infusion. Subjects had a 3-fold range in HDL cholesterol concentration and a 2-fold range in plasma apoA-I concentration. Their mean HDL cholesterol concentration was 1.02 ± 0.09 mmol/l, and their mean plasma apoA-I concentration was 118.4 ± 4.1 mg/dl (Table 1).

Incorporation of labeled leucine into HDL apolipoproteins

Incorporation of deuterated leucine into HDL apolipoproteins was measured as a percentage change in %ttr over the 12 h infusion period (**Fig. 1**). Rate of change in %ttr was linear for all four apolipoproteins. HDL apoE %ttr increased the most rapidly, apoA-I %ttr increased the least rapidly, and apoC-III and apoC-I were intermediate, and were virtually indistinguishable.

HDL apolipoprotein kinetics

Plasma concentration, rate of transport, and RT of HDL apolipoproteins are shown in **Table 2**. Plasma HDL apoE concentration (in mg/dl) was significantly lower than that of HDL apoC-III, which was in turn significantly lower than that of HDL apoC-I, which was in turn significantly lower than that of HDL apoA-I ($P < 0.01$). In molar terms, the concentration profile of HDL apolipoproteins remained the same, such that the concentration of HDL apoE < HDL apoC-III < HDL apoC-I < HDL apoA-I. The average ratio of molecules in the HDL fraction was 1:7:14:60 (i.e., apoE/apoC-III/apoC-I/apoA-I). HDL apolipoprotein TRs were determined by computer analysis (SAAM II) of apolipo-

TABLE 2. Plasma concentration, rate of transport, and residence time of HDL apolipoproteins

	Concentration		Rate of Transport		Residence Time
	mg/dl	*μmol/l*	$mg \cdot kg^{-1} \cdot day^{-1}$	$nmol \cdot kg^{-1} \cdot day^{-1}$	*days*
HDL apoE	2.4 ± 0.2^{a}	0.7 ± 0.1^{a}	1.1 ± 0.1^{a}	31.4 ± 3.3^{a}	$1.1 \pm 0.1^{a,b}$
HDL apoC-III	4.2 ± 0.4^{a}	4.9 ± 0.5^{a}	0.6 ± 0.1^{a}	66.5 ± 9.1^{a}	3.6 ± 0.3^{a}
HDL apoC-I	6.4 ± 0.4^{a}	9.7 ± 0.5^{a}	0.9 ± 0.1^{b}	131.5 ± 12.5^{a}	3.7 ± 0.3^{b}
HDL apoA-I	118.4 ± 4.1^{a}	41.8 ± 1.5^{a}	$11.0 \pm 0.7^{a,b}$	388.6 ± 24.7^{a}	5.1 ± 0.4^{a}

Values represent means ± SE. Data for the four HDL apolipoproteins were compared statistically by paired *t*-test.
^{a,b} Results in the same column sharing the same superscript were significantly different ($P < 0.01$).

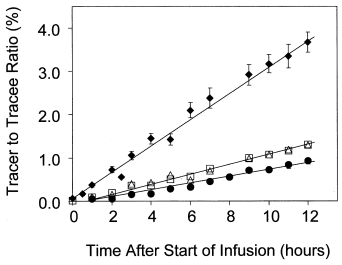

Fig. 1. Enrichment of apolipoprotein E (apoE) (closed diamonds), apoC-III (open squares), apoC-I (open triangles), and apoA-I (closed circles) in HDL with deuterated leucine. Data points represent means ± SEM for 16 subjects. Error bars are too small to be seen for the apoC-III, apoC-I, and apoA-I data points.

protein enrichment curves and were expressed in units of $mg \cdot kg^{-1} \cdot day^{-1}$ and in units of $nmol \cdot kg^{-1} \cdot day^{-1}$. In terms of $mg \cdot kg^{-1} \cdot day^{-1}$, apoA-I had the highest rate of transport and HDL apoC-III had the lowest rate of transport, with HDL apoE and apoC-I being intermediate. In molar terms, however, all four HDL apolipoproteins had significantly different rates of transport ($P < 0.01$), and in magnitude were ordered similarly to HDL molar concentrations (i.e., apoE < apoC-III < apoC-I < apoA-I). In relative terms, the ratio between average molar TRs was 1:2:4:12 (i.e., apoE/apoC-III/apoC-I/apoA-I). The average plasma RT of apoE in HDL was 1.1 days, while the average RT of HDL apoA-I was 5.1 days. The average plasma RTs of HDL apoC-III and apoC-I were very similar (3.6 and 3.7 days, respectively), which were intermediate between HDL apoE and apoA-I. Mean fractional catabolic rates (i.e., the reciprocal of RT) were therefore 0.91, 0.28, 0.27, and 0.20 pools/day for HDL apoE, apoC-III, apoC-I, and apoA-I, respectively.

Relationship between HDL apolipoprotein levels and kinetic parameters

Simple linear regression analysis was carried out to determine which HDL parameters were statistically correlated (**Table 3**). As expected, HDL cholesterol and HDL apoA-I concentrations were significantly correlated ($r = 0.52$, $P < 0.05$). A statistically significant correlation was also observed between HDL apoC-III and HDL cholesterol ($r = 0.66$, $P < 0.01$) and between HDL-apoC-III and HDL apoA-I ($r = 0.72$, $P < 0.01$). The remaining correlation coefficients in Table 3 pertain to the relationship between HDL levels and HDL kinetic parameters (left-hand bottom corner) and between HDL kinetic parameters themselves (bottom right). As expected from previous studies, HDL apoA-I and HDL cholesterol levels were significantly correlated with HDL apoA-I RTs ($r = 0.56$ and $r = 0.69$), and were not significantly correlated with HDL apoA-I TRs. This result is shown graphically in **Fig. 2**. In contrast, HDL apoE, apoC-III, and apoC-I levels were all positively related to their TRs and not their RTs. Positive relationships between TRs and HDL apoE, apoC-III, and apoC-I levels ($r = 0.66$, $P < 0.01$; $r = 0.73$, $P < 0.01$; and $r = 0.62$, $P < 0.05$, respectively), are shown in Fig. 2, contrasting to data for apoA-I. Importantly, HDL apoC-III TRs were postively correlated with both HDL cholesterol and apoA-I levels ($r = 0.54$ and $r = 0.53$, $P < 0.05$, respectively), and HDL apoC-III TRs were in turn related to HDL apoA-I RTs ($r = 0.51$, $P < 0.05$).

DISCUSSION

The present study has allowed for a direct comparison to be made between the plasma concentration and kinetics of four HDL apolipoproteins: apoA-I (the principle structural protein of HDL), and apoC-I, apoC-III, and apoE (three apolipoproteins that can transfer or exchange between TRL and HDL). Our results show that in terms of mass, there was approximately two times more apoC-III than apoE in the HDL fraction isolated by ultracentrifugation. There was approximately three times more apoC-I than apoE, and nearly 50× more apoA-I than apoE. Taking into account the different molecular weights of these proteins, the molar ratio in the HDL fraction was 1:7:14:60 (apoE/apoC-III/apoC-I/apoA-I). In obtaining these ratios, it is important to note that we have assumed

TABLE 3. Relationship between plasma HDL concentrations and kinetic parameters

	HDL chol	HDL apoA-I	HDL apoE	HDL apoC-III	HDL apoC-I	HDL apoA-I TR	HDL apoA-I RT	HDL apoE TR	HDL apoE RT	HDL apoC-III TR	HDL apoC-III RT	HDL apoC-I TR	HDL apoC-I RT
HDL chol													
HDL apoA-I	0.52[a]												
HDL apoE	0.18	0.16											
HDL apoC-III	0.66[b]	0.72[b]	0.16										
HDL apoC-I	0.32	0.44	0.29	0.34									
HDL apoA-I TR	−0.44	0.10	−0.14	−0.09	0.38								
HDL apoA-I RT	0.69[b]	0.56[a]	0.19	0.61[a]	−0.09	−0.75[c]							
HDL apoE TR	0.21	−0.04	0.66[b]	0.17	0.48	−0.30	0.20						
HDL apoE RT	−0.12	0.12	0.25	−0.07	−0.27	0.30	−0.17	−0.53[a]					
HDL apoC-III TR	0.54[a]	0.53[a]	0.21	0.73[b]	0.24	−0.17	0.51[a]	0.37	−0.32				
HDL apoC-III RT	−0.09	0.02	−0.05	−0.03	−0.02	0.12	−0.03	−0.29	0.41	−0.66[b]			
HDL apoC-I TR	0.50	0.06	0.18	0.10	0.62[a]	0.04	−0.07	0.35	−0.29	0.16	−0.22		
HDL apoC-I RT	−0.48	0.17	−0.19	0.04	−0.06	0.37	−0.14	−0.18	0.04	−0.02	0.14	−0.77[c]	

RT, residence time; TR, transport rate. Values represent correlation coefficients (r).
[a] Significantly correlated: $P < 0.05$.
[b] Significantly correlated: $P < 0.01$.
[c] Significantly correlated: $P < 0.001$.

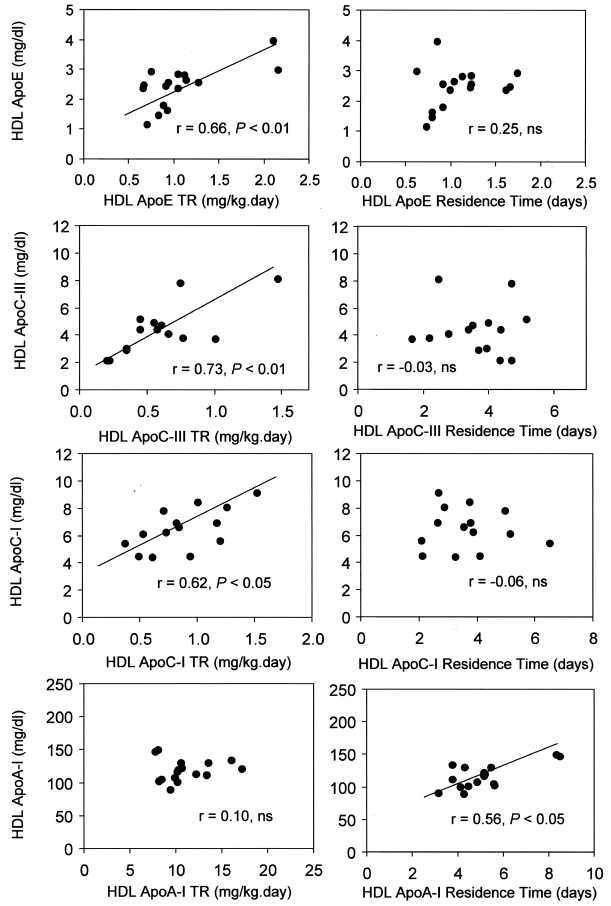

Fig. 2. Relationship between plasma HDL apolipoprotein concentrations and their rates of transport and residence times (RTs). Data that were significantly correlated ($P < 0.05$) have linear regression lines. ns, not significant.

that any apoC-I, apoC-III, or apoE in the d > 1.21 g/ml fraction was HDL apolipoprotein. For apoC-I and apoC-III this represented less than 3% of total plasma; however, for apoE there was 14.6 ± 0.9% of total apoE in the bottom fraction. It is generally accepted that this represents apoE that is stripped away from lipoproteins during sequential ultracentrigation (33, 34). As we have proposed previously (25), there are at least three reasons to support the fact that apoE in the d > 1.21 g/ml fraction should be regarded as HDL apoE: *1*) fast protein liquid chromatography separation of plasma lipoproteins (which avoids the high sheer stress of ultracentrifugation and therefore does not result in dissociation of apoE from lipoproteins) provides an HDL apoE concentration of 2.3 ± 0.3 mg/dl for normolipidemic (NL) subjects (n = 12) and 2.0 ± 0.3 mg/dl for combined hyperlipidemic (CHL) subjects (n = 12) (32). Combining HDL and d > 1.21 g/ml apoE gives similar concentrations for HDL apoE (i.e., 2.9 ± 0.3 mg/dl for NL subjects and 2.1 ± 0.3 mg/dl for CHL subjects) (25). *2*) When apoE was isolated from the d > 1.21 g/ml fraction by affinity chromatography in a single untreated patient, its kinetics were found to resemble those of HDL apoE rather than VLDL apoE (25). *3*) Percentage recovery of apoE in the bottom fraction tends to be lower in hypertriglyceridemic (HTG) versus NL subjects, despite the fact that the percentage of plasma apoE associated with TRL is 2- to 3-fold higher in HTG subjects. ApoA-I can also be stripped from HDL by ultracentrifugation (35), and for this reason we have taken total plasma apoA-I concentration to represent apoA-I in HDL.

Assuming that there are two to four molecules of apoA-I in each HDL particle, the aforementioned data suggest that less than 10% of all HDL particles in the circulation contain apoE, less than half contain apoC-I, and about two-thirds contain apoC-III. These apolipoproteins are not evenly distributed among apoA-I-containing particles because, for example, apoE is found in larger (9–18.5 nm) particles, which tend to have slow preβ migration on agarose gels. ApoC-III on the other hand, like apoA-II, is found in smaller (5–12 nm) α-migrating HDL (36). Some HDL particles evidently contain more than one exchangeable apolipoprotein, or alternatively more than one copy of apoC-I, apoC-III, and/or apoE. In view of this particle heterogeneity, it is not surprising that HDL apolipoproteins have significantly different rates of transport and significantly different RTs (Table 2). In molar terms, apoE had the lowest and apoA-I had the highest TR, and the ratio between TRs was 1:2:4:12 (apoE/apoC-III/apoC-I/apoA-I). Each molecule of apoE remained in the HDL fraction for an average of one day, while each molecule of apoA-I remained in HDL for an average of 5 days. ApoC-III and apoC-I on the other hand had intermediate RTs between 3.5 and 4 days. As reviewed by Marsh, Welty, and Schaefer (37), 10 previous stable-isotope studies together provided a weighted average of 11.5 ± 2.8 $mg \cdot kg^{-1} \cdot day^{-1}$ for HDL apoA-I TR and 0.206 ± 0.043 pools/day for apoA-I fractional catabolic rate (equivalent to an RT of 4.85 days). These data compare very well with those obtained for apoA-I in the present study (i.e., HDL apoA-I TR: 11.0 ± 0.7 $mg \cdot kg^{-1} \cdot day^{-1}$ and HDL apoA-I RT: 5.1 ± 0.4 days). Our data for HDL apoC-I also compare well with the only other previous apoC-I kinetic study by Malmendier et al. (38). ApoC-I does not contain tyrosine residues and cannot be labeled conventionally with radioactive iodine. This problem was overcome by labeling apoC-I with Bolton and Hunter (BH) reagent. More than 80% of injected ^{125}I-BH-apoC-I was found associated in plasma with HDL (38), and for four NL subjects, apoC-I TR was determined to be 1.8 $mg \cdot kg^{-1} \cdot day^{-1}$ and RT was 3.2 days, which compares to 0.9 ± 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ and 3.7 ± 0.3 days, respectively, in the present study. As far as apoE is concerned, a recent study by Hannuksela et al. (39) reported RTs of 0.60, 0.47, and 0.38 days for ^{125}I-labeled apoE in LpE:A-I:A-II, LpE:A-II, or LpE:A-I particles injected into normolipidemic subjects. These RTs are somewhat less than the 1.1 ± 0.1 days for HDL apoE in this study, which may reflect the fact that ^{125}I-labeled apoE can exchange or transfer more readily from HDL to VLDL than endogenously labeled apoE. It is certainly striking that the plasma kinetics of endogenously-labeled apoC-I, apoC-III, and apoE are quite different when isolated from HDL rather than VLDL. Rate of appearance of deuterated apoC-I, apoC-III, and apoE is consistently faster in VLDL

than HDL (24–26), suggesting that in endogenous-labeling experiments carried out in the fasted state, there is only very slow apolipoprotein exchange or transfer between these fractions. Alternatively, there are exchangeable and nonexchangeable pools of HDL and VLDL apolipoproteins, with exchangeable pools being relatively small compared with the nonexchangeable ones. The presence of exchangeable and nonexchangeable pools of apoC-III and apoE has been proposed previously by Bukberg et al. (40) and Gregg et al. (41, 42).

An interesting feature of the present data is that HDL apoA-I concentration was significantly correlated with RT and not with TR, while levels of HDL apoC-I, apoC-III, and apoE were significantly correlated with their TRs and not their RTs (Fig. 2). These data suggest that catabolism rather than production is the major determinant of HDL apoA-I levels, as suggested by previous studies (43–45). In contrast, production rather than catabolism is the major determinant of plasma levels of HDL apoC-I, apoC-III, and apoE. Production, as measured by rate of transport in the present study, could represent: *a*) direct secretion of apoA-I-containing particles by the liver or intestine containing apoC-I, apoC-III, and/or apoE; *b*) synthesis of lipid-poor apoC-I, apoC-III, and/or apoE, which associates with cell membranes and is transferred to circulating HDL; *c*) synthesis and secretion of apoC-I, apoC-III, and/or apoE on VLDL that is transferred or transformed into HDL; or *d*) a combination of the above. Irrespective of the exact mechanism, it is clear that the plasma concentration of different HDL apolipoproteins is regulated by different mechanisms and that HDL apolipoproteins are kinetically as well as dynamically different.

A final significant feature of the present study is the consistent positive correlations that were observed between HDL apoC-III and apoA-I metabolic parameters. For the sake of clarity, these statistically significant relationships ($P < 0.05$) are presented diagramatically in **Fig. 3**. First, HDL apoC-III levels were found to be positively correlated with those of HDL cholesterol and HDL apoA-I. Second, HDL apoC-III TRs were correlated with HDL cholesterol, HDL apoC-III, and HDL apoA-I. Finally, a positive relationship was found between HDL apoC-III TRs and HDL apoA-I RTs, meaning that HDL apoC-III production was inversely correlated with HDL apoA-I catabolism. Similar relationships were not observed for HDL apoC-I or HDL apoE. Plasma levels of HDL were therefore more strongly linked to the metabolism of HDL apoC-III than to HDL apoC-I or HDL apoE. This is not to say that apoC-I and apoE do not have significant roles to play in HDL metabolism, but simply that the link between apoC-III metabolism and HDL was the most evident in vivo. We favor the view that this represents an effect of apoC-III on HDL catabolism and hence HDL levels, rather than an effect of HDL levels on apoC-III concentration and production. One possibility is that increased production of HDL apoC-III and/or increased flux of apoC-III into HDL results in increased levels of HDL apoC-III and subsequent inhibition of HDL catabolism. This is supported by the data of Luc et al. (46), showing that in 489

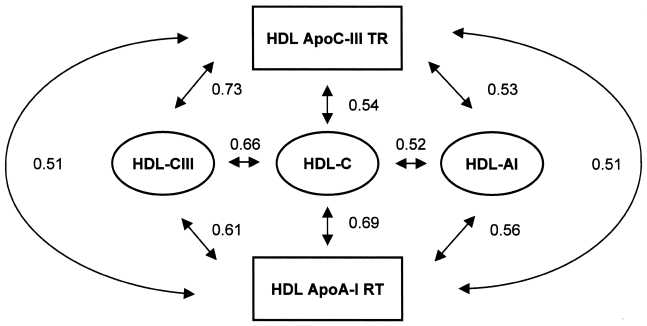

Fig. 3. Diagram showing statistically significant relationships ($P < 0.05$), defined by r values, between HDL apoC-III transport rates, HDL apoA-I RTs, and plasma concentrations of HDL apoC-III, HDL cholesterol, and HDL apoA-I.

control subjects without CHD not taking hypolipidemic drugs, apoC-III-Lp nonB (measured by electroimmunassay after immunoprecipitation of apoB-containing lipoproteins) was significantly correlated with HDL cholesterol and apoA-I concentrations. ApoE-Lp nonB on the other hand was only weakly correlated with HDL cholesterol and apoA-I. Sacks et al. have similarly demonstrated a weak but statistically significant correlation between HDL apoC-III and HDL cholesterol levels in a subgroup of patients (n = 788) enrolled in the Cholesterol and Recurrent Events trial (47). Second, Stephan, Gibson, and Hayes (48) have shown in cebus monkeys that the rate of removal of cholesteryl ester from HDL was proportional to the apoE to apoC-III ratio of these particles. Increased amounts of apoC-III in HDL were thus associated with reduced HDL cholesteryl ester removal. Finally, Le, Gibson, and Ginsberg (49) demonstrated in a group of 19 subjects that concentrations of apoC-III and apoA-I in HDL were significantly correlated ($r = 0.73$, $P < 0.005$) and that there was an inverse relationship between HDL apoC-III concentration and the FCR for apoA-I ($r = -0.67$, $P < 0.005$). Together, these data support the concept that apoC-III may play an important role in regulating HDL catabolism, a concept that deserves further investigation in isolated tissues and cultured cells.

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