Mass kinetics of apolipoprotein A-I in interstitial fluid after administration of intravenous apolipoprotein A-I/lecithin discs in humans

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Abstract Apolipoprotein kinetics are customarily determined by modeling time curves of specific radioactivity or isotopic enrichment in plasma after intravenous infusion of radiolabeled lipoproteins or stable isotope-enriched amino acids. However, this provides no information on the fractional rate of transfer of the apolipoprotein from plasma to interstitial fluid ($k_{p\text{-}if}$) or its mean residence time in interstitial fluid (MRT_{if}). To determine these parameters for a pharmacologic dose of exogenous apolipoprotein A-I (apoA-I) given intravenously as apoA-I/lecithin discs, we measured apoA-I in plasma and prenodal leg lymph in five healthy men before, during, and after a 4 h infusion at 10 mg/kg/h. ApoA-I concentrations in plasma and lymph were modeled by linear compartmental models (SAAM II version 1.1), using lymph albumin to adjust for the effects of variations in lymph flow rate. $k_{p\text{-}if}$ averaged 0.75%/h (range, 0.33–1.32), and MRT_{if} averaged 29.1 h (14.1–40.0). Neither parameter was correlated with the distribution volume (57–105 ml/kg) or the fractional elimination rate (1.44–2.91%/h) of apoA-I, determined by modeling plasma apoA-I concentration alone. ■ Although used here to study the mass kinetics of apoA-I, if combined with infusion of a tracer, analysis of lymph could also expand the modeling of endogenous apolipoprotein kinetics.—Hovorka, R., M. N. Nanjee, C. J. Cooke, I. P. Miller, W. L. Olszewski, and N. E. Miller. **Mass kinetics of apolipoprotein A-I in interstitial fluid after administration of intravenous apolipoprotein A-I/ lecithin discs in humans.** *J. Lipid Res.* 2006. **47:** 975–981.

Supplementary key words high density lipoprotein • interstitium • lipoprotein • lymph • tissue fluid

Apolipoprotein kinetics are customarily determined either by modeling the plasma specific radioactivity-time curve after intravenous injection of radioiodinated lipoprotein or by infusing a stable isotope-enriched amino acid and modeling its appearance in the apolipoprotein in plasma by mass spectrometry. Both procedures have provided much valuable information (1–5). However, a limitation of methods that rely on plasma measurements alone is their inability to provide information on either the fractional rate of transfer of the apolipoprotein across the endothelium from plasma to the interstitial fluid (IF) or its residence time in IF. Such data might improve our understanding of lipoprotein metabolism in the interstitium, such as the oxidative modification of low density lipoproteins (6) and reverse cholesterol transport by HDLs (7). To obtain such information, it is necessary to model changes in both plasma and IF.

We have previously shown that IF can be collected continuously for several days from ambulant humans via a cannula placed in an afferent (prenodal) lymph vessel of the leg (8). We have now applied this technique to study the pharmacokinetics of exogenous apolipoprotein A-I (apoA-I) in IF after intravenous infusion of apoA-I/lecithin discs, a procedure of current interest as a potential new therapy for coronary heart disease (9, 10).

METHODS

Subjects

Five healthy men with low-normal plasma total and HDL cholesterol concentrations were studied (**Table 1**). All had undergone physical examination, electrocardiography, routine clinical chemistry and hematology, and screening for recreational drugs. None had evidence of renal, hepatic, endocrine, or cardiovascular disease or of drug abuse. None was taking a special diet or medication. The study had been approved by the relevant ethics committee. All subjects had given written informed consent.

Abbreviations: apoA-I, apolipoprotein A-I; IF, interstitial fluid; $k_{p\text{-}if}$, fractional rate constant for transfer from plasma to interstitial fluid; MRT_{if}, mean residence time in interstitial fluid.
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TABLE 1. Clinical details of the study subjects

	Subject number						
Variable	1	2	3	4	5	Mean	SEM
Age (years)	27	22	28	25	21	24.6	1.36
Weight (kg)	78	78	86	75	77	78.8	1.88
Body mass index (kg/m^2)	23.3	24.9	25.9	21.6	25.3	24.2	0.78
Plasma cholesterol (mmol/l)	3.36	3.87	3.86	3.23	3.10	3.48	0.16
Plasma triglycerides (mmol/l)	1.13	1.50	1.34	1.22	0.57	1.15	0.16
HDL cholesterol (mmol/l)	0.86	1.09	1.10	1.00	1.05	1.02	0.04
ApoA-I (mg/dl)	67	78	71	79	104	80	6.4

ApoA-I, apolipoprotein A-I. Measurements were made on the day after admission to the ward. Lipids and apoA-I were quantified in fasting blood (14 h overnight).

Clinical procedures

On day 1, subjects were admitted to a metabolic ward (London Bridge Hospital) and placed on an isocaloric solid food diet (cholesterol, 188 mg/d; total fat, 65 g/d; saturated-polyunsaturated-monounsaturated fatty acids, 44:22:34% weight; fat-protein-carbohydrate, 21:20:59% calories). On day 4, a fine polyethylene cannula (Intramedic® tubing PE60; Becton Dickinson and Co., Sparks, MD; inner diameter, 0.76 mm; outer diameter, 1.22 mm) was inserted into an afferent lymph vessel ∼6 cm above the ankle (8). The external end of the cannula was passed into a 3 ml plastic tube containing 2 mg of solid Na$_2$EDTA, which was lightly strapped to the leg. The procedure was carried out under local anesthesia and sterile conditions. Volunteers were then transferred to the metabolic ward. Thereafter, lymph was collected continuously until the end of the study, during which the subjects were ambulant, adhered to the diet, and avoided alcohol.

On day 7, at ∼9:00 AM, a cannula was placed in an antecubital vein for blood sampling and kept patent by intermittent flushing with sterile 0.15 mol/l NaCl. Commencing 1 h later, a 4 h intravenous infusion of apoA-I/lecithin discs (ZLB Behring, Bern, Switzerland) was given via an antecubital vein of the other arm (10 mg apoA-I/kg/h, 0.4 ml/kg/h; total dose, 40 mg/kg) (11). The physical and chemical properties of the discs have been described (11). Every 3 or 4 h thereafter, a 5 ml blood sample was collected into Na$_2$EDTA (final concentration, 1 mg/ml), and the lymph collection tube was replaced with a new one. This was continued for 72–144 h from the start of the infusion.

Body weights were measured daily and remained essentially constant. No clinical complications developed, and no abnormalities of clinical chemistry or hematology were recorded.

Laboratory procedures

Blood and lymph tubes were kept on ice before centrifugation at 4°C (2,500 g, 20 min). Multiple aliquots of plasma and supernatant lymph were transferred to polypropylene tubes, which were kept at −80°C until analysis. Total lymph volume in each tube was determined by weighing before and after removal of lymph.

Plasma cholesterol and triglycerides were quantified using commercial enzymes (Sigma, Poole, UK) and Trinder-type reagents (Research Organics) in a microtiter plate spectrophotometer (11). Precinorm L® (Boehringer-Mannheim GmBH, Mannheim, Germany) was used as a calibrator. Plasma HDL cholesterol was quantified after precipitation of apoB-containing lipoproteins with polyethylene glycol 6000 [8%, w/v (final concentration)] (11). ApoA-I in plasma and lymph was quantified by rocket immunoelectrophoresis in the presence of polyethylene glycol 6000 (3%, w/v) and Tween 20 (0.2%, w/v) (11). The primary antiserum was goat polyclonal IgGs raised against

delipidated human apoA-I (International Immunology Corp.). ApoA-I assays were standardized using dilutions of Precinorm L. Albumin concentrations in plasma and lymph were quantified by immunoelectrophoresis (11). Coefficients of variation for all immunoassays were <10%. Plasma and lymph samples from the same subject were processed together. All assays were done in duplicate, and mean values were calculated.

Data analysis and modeling

The durations of the experiments used for modeling were 192, 124, 156, 192, and 120 h for subjects 1–5, respectively. All modeling was done using the SAAM II program version 1.1 (SAAM Institute, Seattle, WA). It was assumed that the infusion of apoA-I had no effect on endogenous apoA-I synthesis or catabolism and that the kinetics of apoA-I were time-invariant during the study.

First, the changes in plasma apoA-I concentration alone were modeled, as described previously (12), to provide the distribution volume of the infused apoA-I (V) and the fractional rate constant for elimination (k_{el}). The model is described by the following equations:

$$dc_P(t)/dt = -k_{el} \times [c_P(t) - c_{P0}] + U(t)/V$$

$$c_{P0} = c_P(0)$$

where $c_P(t)$ is the plasma apoA-I concentration, c_{P0} is the plasma apoA-I concentration before the start of the apoA-I infusion (calculated as the average concentration at −4, −2, and 0 h relative to the start of the infusion), and $U(t)$ is the apoA-I infusion rate. The time origin coincides with the start of the apoA-I infusion.

Second, apoA-I concentrations in plasma and lymph were modeled simultaneously to provide the fractional rate of transfer of apoA-I from plasma to IF ($k_{p\text{-}if}$) and the mean residence time of apoA-I in IF (MRT$_{if}$). ApoA-I concentrations in lymph were adjusted for the effect of variations in lymph flow rate by entering lymph albumin concentration as an independent variable (13). A two-compartment model was used, composed of a single pool for plasma apoA-I and a single pool for IF apoA-I. Transfer from plasma to IF was assumed to be linearly dependent on plasma apoA-I concentration. The disappearance of apoA-I from IF represented its return to plasma and any degradation by peripheral cells. The model is described by the following equations:

$$dc_l(t)/dt = -k_{dis} \times c_l(t) + k_{p\text{-}if} \times c_P(t)$$

$$c_{L0} = c_l(0)$$

$$c_{LC}(t) = c_l(t) \times a(t)/a_0$$

$$MRT_{if} = 1/k_{dis}$$

where $c_l(t)$ is the apoA-I concentration in lymph, c_{L0} is the lymph apoA-I concentration at the start of the experiment, $c_P(t)$ is the plasma apoA-I concentration, $c_{LC}(t)$ is the lymph apoA-I concentration corrected for lymph flow rate, $a(t)$ is the lymph albumin concentration, a_0 is the average lymph albumin concentration during the experiment, and k_{dis} is the fractional disappearance rate of apoA-I from IF. The time origin was taken as the first measurement of lymph apoA-I concentration.

The kinetic parameters of exogenous apoA-I were estimated using a nonlinear, weighted, least-squares algorithm. The weight was defined as the reciprocal of the square of the nominal measurement error, with a coefficient of variation of 10%. The precision of the parameter estimates was obtained from the Fisher information matrix and expressed as coefficient of variation (14). For the rate constant for elimination, these were all <18% (mean, 11.4%); for distribution volume, they were <11% (mean, 7.6%); and with one exception (subject 5), they were <35% (mean, 25.0%) for $k_{p\text{-}if}$ and <38% (mean, 25.3%) for MRT_{if}. In subject 5, precision was 81% for $k_{p\text{-}if}$ and 83% for MRT_{if}. A value of <100% is regarded as acceptable for such parameter estimates (14).

In addition to calculation of the precision of parameter estimates, model validation included assessment of model fit using a plot of weighted residuals and use of the Runs test to evaluate the random distribution of residuals (14).

RESULTS

Plasma apoA-I concentration increased on average by 62.2 mg/dl during the infusion and returned to baseline by 4.5 days (range, 3.0–5.8 days). As in our other studies (11), this was accompanied by rapid but transient increases in plasma HDL unesterified cholesterol and preβ apoA-I concentrations and slower increases in plasma HDL cholesteryl ester and α apoA-I concentrations (data not shown).

Plasma apoA-I, lymph apoA-I, and lymph albumin concentrations in all subjects are shown in **Fig. 1**. Lymph apoA-I/albumin ratios are also shown. As documented previously (13), lymph apoA-I and albumin showed circadian variation, fluctuating inversely with lymph flow rate
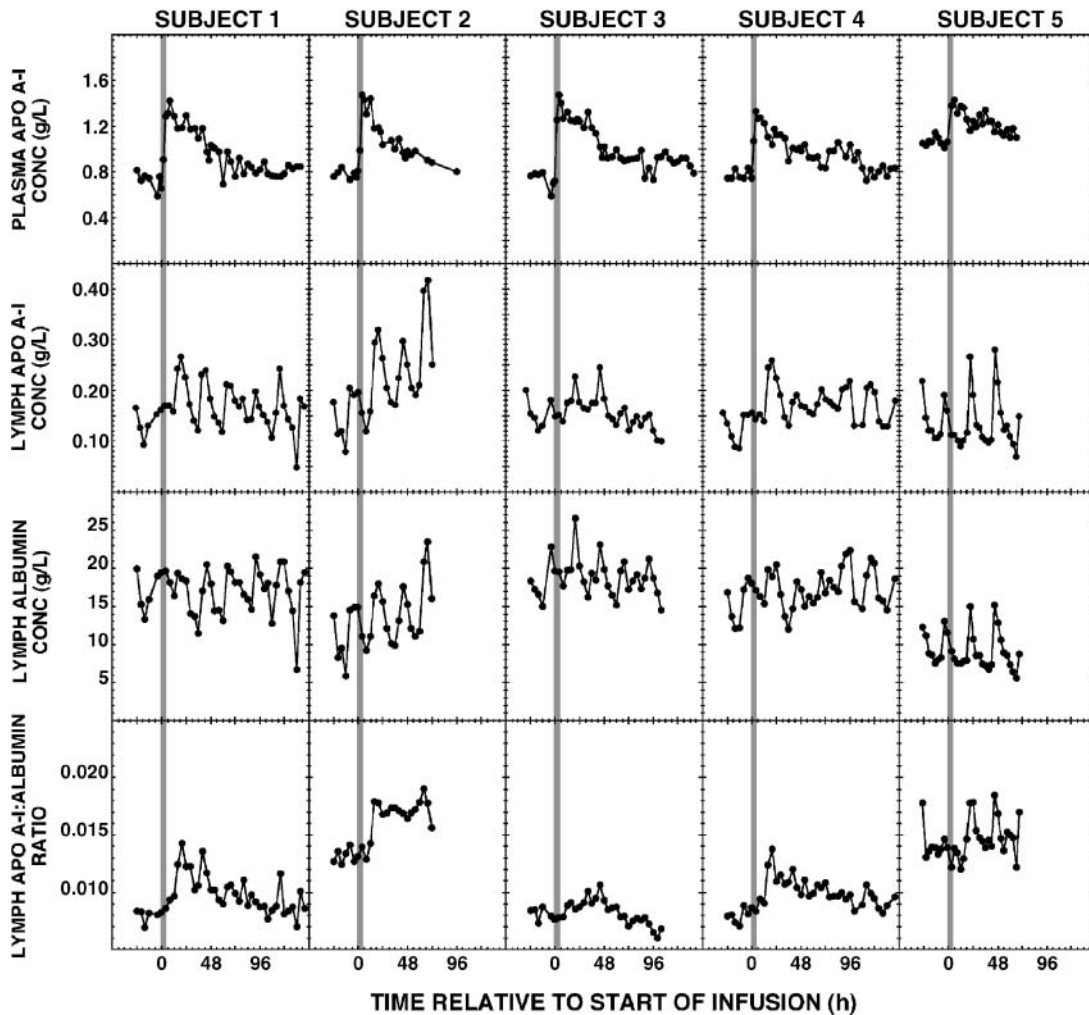

Fig. 1. Plasma apolipoprotein A-I (apoA-I) concentrations, lymph apoA-I concentrations, lymph albumin concentrations, and lymph apoA-I/albumin ratios in each subject before, during, and after intravenous infusion of apoA-I/lecithin discs (10 mg/kg/h for 4 h). The infusion period is shown by the vertical shaded bars. Plasma albumin concentration was unchanged (not shown).

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TABLE 2. Parameters of apoA-I disposition in plasma and IF after apoA-I/phosphatidylcholine infusion

Variable	Subject number					Mean	SEM
	1	2	3	4	5		
Change in plasma apoA-I (mg/dl)	78	68	77	50	38	62	7.9
Distribution volume (ml/kg)[a]	57	61	59	80	105	72.4	9.1
Fractional rate constant for elimination (%/h)[a]	1.69	2.91	1.44	2.15	2.27	2.09	0.25
Fractional rate constant for plasma-to-IF transfer (%/h)[b]	1.32	0.56	0.40	1.12	0.33	0.75	0.20
Mean residence time in IF (h)[b]	14.1	40.0	38.8	15.8	36.9	29.1	5.8

IF, interstitial fluid.
[a] Determined from plasma concentration-time curve.
[b] Determined from plasma and lymph concentration-time curves.

(i.e., with IF production rate). Average lymph flow rates in subjects 1–5 were 7.4, 24.6, 1.2, 3.4, and 2.5 μl/min, respectively. In the pooled data, flow rate was unaffected by the infusion of discs (ANOVA, $P = 0.40$). Plasma albumin concentration was also unchanged (data not shown).

The parameter estimates are given in **Table 2**. Modeling of plasma apoA-I concentration alone yielded a distribution volume of 72.4 ± 9.1 ml/kg (mean ± SEM) and a fractional rate constant for elimination of 2.09 ± 0.25%/h. Modeling of plasma and lymph apoA-I simultaneously gave values of 0.75 ± 0.20%/h for the rate constant for plasma-to-IF transfer and 29.1 ± 5.81 h for the mean residence time in IF. There was a trend for the mean residence time to increase with increasing plasma HDL cholesterol (Spearman $r = 0.90$, $P = 0.08$). The increment in plasma apoA-I during the infusion was not significantly correlated with any of the kinetic parameters.

Model validity was supported by the distribution of weighted residuals. Visual assessment indicated that mean weighted residuals displayed a random pattern around −1 to +1, with standard deviations mostly crossing the zero line (**Fig. 2**). The weighted residuals associated with the model of lymph apoA-I kinetics displayed a more rugged pattern than those of plasma, with two mean weighted residuals and associated standard deviations being outside the −1 to +1 interval. This can be explained by the greater chance of measurement error associated with the model of lymph apoA-I kinetics, attributable to the involvement of three measurement processes (plasma apoA-I, lymph apoA-I, and lymph albumin) compared with one measurement process (plasma apoA-I) in the model of plasma apoA-I kinetics. The Runs test supported the random distribution of residuals, with the exception of the plasma apoA-I model in subject 5 ($P < 0.05$, after Bonferroni correction for multiple comparisons) and the lymph apoA-I model in subject 3 ($P < 0.05$).

DISCUSSION

Because afferent peripheral lymph is essentially identical in composition to IF (15, 16), our results represent the kinetics of apoA-I in the interstitium. Although the advantages of sampling from more than one compartment are well recognized, this is the first time that long-term collection of lymph has been used to study the kinetics of a protein in humans or those of an apolipoprotein in any species. The only comparable studies have been of the kinetics of albumin in animals (17). Engeset et al. (18) described the appearance of radioiodinated albumin in leg lymph in eight cancer patients, but they did not model the data. Bergan et al. (19) modeled the concentrations of antibiotics in human leg lymph, but only for 12 h. For our purposes, we needed to collect for a longer period.

In all subjects, lymph apoA-I concentration decreased during the day and increased at night. Such circadian variations occur in all lymph protein concentrations in ambulant subjects, as a consequence of the alterations in lymph flow rate (i.e., IF production rate) that are pro-
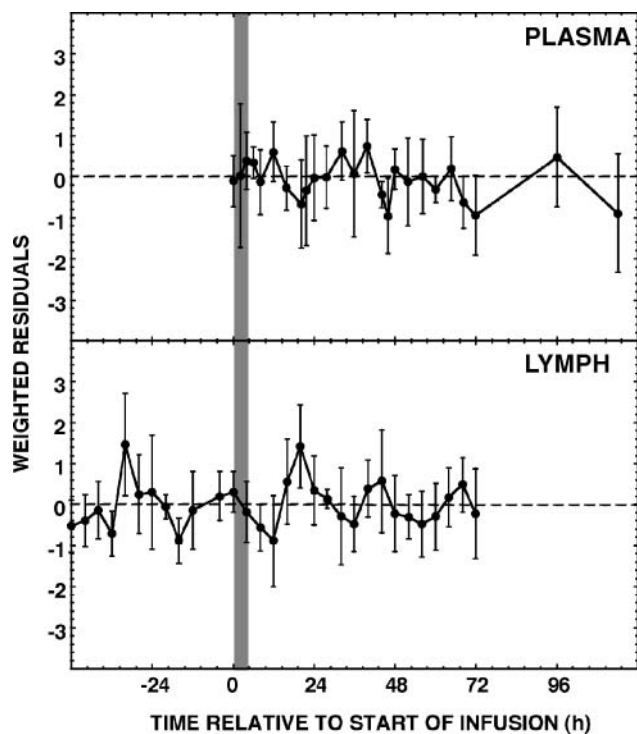

Fig. 2. Weighted residuals (means ± SD) associated with the models of plasma apoA-I and lymph apoA-I kinetics. Weighted residuals have no units.

duced by changes in posture and physical activity (13, 15). The amplitude of the fluctuation varies from protein to protein as a function of molecular size. We have shown that lymph apoA-I concentration can be adjusted for this effect by reference to lymph albumin concentration (13).

Our model assumed that plasma apoA-I and IF apoA-I behave as two distinct pools. Studies of endogenous apoA-I kinetics, involving sampling of plasma alone after infusion of tracers, have also used a two-pool model, assumed to represent intravascular and extravascular compartments (20–25). Although recent work has shown that apoA-I in subclasses of plasma HDLs is actually kinetically heterogeneous, with preβ apoA-I turning over faster than α apoA-I (26), this is unlikely to have introduced a large error into our estimates, owing to the much greater pool size of α apoA-I.

Our model also assumed that the infusion did not perturb the metabolism or kinetics of endogenous apoA-I. There is no evidence that apoA-I synthesis in liver or intestine is affected by plasma apoA-I concentration. It is well documented that the fractional elimination rate and the plasma concentration of apoA-I are negatively correlated (20–25). Although this is owing at least in part to an effect of the former on the latter, the question arises whether a primary increase in concentration decreases the fractional elimination rate. The available evidence suggests that it does not. When Doran (27) infused apoA-I/lecithin discs into rats (n = 10) and dogs (n = 4) at different doses over the ranges 120–600 mg/kg and 100–300 mg/kg, respectively, the fractional elimination rate was unchanged. When we infused the same discs at doses of 25 and 40 mg apoA-I/kg into healthy men with similar baseline apoA-I concentrations (n = 3 and 4, respectively), plasma apoA-I increased by 27 and 52 mg/dl on average, but the concentration decline thereafter was similar visually in the two groups (11). We have now modeled these data using the same modeling procedure used in the present work. The results confirmed that the fractional removal rates did not differ significantly at the two doses (2.48 ± 0.66%/h and 2.64 ± 0.47%/h at the low and high doses, respectively).

On the basis of our current understanding of the mechanisms by which macromolecules cross endothelium from plasma, it is unlikely that the infusion will have altered the fractional rate of transfer of apoA-I to IF. In contrast to small molecules, which cross endothelia bidirectionally by diffusion, proteins move from plasma to IF by convection (solvent drag), as a consequence of the flow of water through pores at cell junctions, and return to plasma exclusively via the lymphatic system (15, 28). As the infusion had no effect on lymph flow rate, there must also have been no effect on water filtration rate. Under such conditions, the flux of a macromolecule to IF is linearly related to its concentration in plasma (15, 28). We have shown that, at a constant flow rate (e.g., samples collected at the same time of day, or under standardized conditions of posture and physical exercise), the concentration of apoA-I in lymph is directly related to its concentration in plasma (8, 13).

Because we have shown that apoA-I/lecithin discs interact with plasma HDLs in vivo to increase the proportion of apoA-I in small preβ HDLs and the size of α HDLs (11, 29), the question arises whether these changes could have affected the movement of apoA-I across the endothelium. Although this possibility cannot be discounted, any affect is likely to have been small, because the changes in HDL subclasses are relatively short-lived (11). Furthermore, although an increase in the number of preβ HDLs (smaller than α HDLs) would be expected to increase the rate of transfer of apoA-I across the endothelium, enlargement of α HDLs would have the opposite effect.

Our model also assumed that the disappearance of apoA-I from IF represents its return to plasma via lymph plus any degradation by peripheral cells. It is generally accepted that macromolecules return from IF to blood exclusively via the lymphatic system (15, 30), and not via venous capillaries. Degradation of apoA-I by peripheral cells makes a small contribution to total apoA-I catabolism, which occurs mostly in the liver and kidney (31).

Our data are of interest in relation to the use of apoA-I/lecithin discs in the treatment of coronary atherosclerosis (10). In the only clinical trial reported to date (9), apoA-I$_{Milano}$/lecithin discs were given at doses of 15 or 45 mg/kg at intervals of 1 week for 5 weeks. To our knowledge, there have been no comparisons of the concentrations of lipoproteins in peripheral tissue IF and artery wall IF in any species. If our present findings are representative of the latter, they suggest that native apoA-I/lecithin discs may need to be given more often than once weekly if they are to expose artery wall cells continuously to significantly

TABLE 3. Calculated values for priming dose, subsequent constant infusion rate, and time taken to produce a steady-state 2-fold increase in apoA-I concentration in IF in each subject

	Subject number						
Variable	1	2	3	4	5	Mean	SEM
Priming dose (mg/kg)	38	47	42	63	108	59.6	12.8
Infusion rate (mg/kg/h)	0.6	1.4	0.6	1.4	2.5	1.3	0.35
Time to reach 95% steady-state concentration in IF (h)	42	120	116	47	111	87.2	17.5

Formulae used were as follows: $D = \Delta c_P \times V$, $U = \Delta c_P \times V \times k_{el}$, and $t_{95\%} = -\ln(0.05) \times MRT_{if}$, where D is the priming dose, Δc_P is the increment in plasma apoA-I (mg/ml), V is the volume of distribution (ml/kg), U is the infusion rate required to maintain Δc_P, k_{el} is the fractional rate constant for elimination (1/h), $t_{95\%}$ is the time taken to reach 95% of the steady-state concentration in IF, and MRT_{if} is the mean residence time of apoA-I in IF (h).

increased concentrations of apoA-I. Knowledge of the parameters of apoA-I kinetics in IF could assist the design of alternative therapeutic regimens, as they enable the concentration-time curves in both plasma and IF to be predicted at different doses and infusion rates. For a hypothetical regimen consisting of a priming bolus followed by a constant infusion, **Table 3** presents the doses and infusion rates that would maintain 2-fold increases in plasma and IF concentrations in the five subjects, together with the times taken to achieve those concentrations in IF.

Because we used a pharmacologic dose of exogenous apoA-I, our results do not permit any conclusions to be drawn about the kinetics of endogenous apoA-I. Such information would be of physiologic interest in relation to the dynamics of reverse cholesterol transport (7, 29), lipoprotein oxidation (6), and lipoprotein remodeling (32) in peripheral IF. For that purpose, further work will be required, in which lymph collection is combined with infusion of a radioactive or stable isotopic tracer. Such studies would be less complex, as they would not require any assumptions to be made about the effects of changes in apoA-I pool size or HDL subclass distribution. Nor would adjustments be required to correct for the effects of variations in lymph flow rate, because these would not be expected to alter the specific radioactivity or stable isotope enrichment of apoA-I in lymph.

The values we obtained for the fractional elimination rate of exogenous apoA-I mass (1.44–2.91%/h) were somewhat greater than most published values for endogenous apoA-I measured using tracers (0.4–1.8%/h) (20–25). It is not clear whether this reflects a difference between exogenous apoA-I and endogenous apoA-I or is attributable to the fact that the plasma HDL concentrations in our subjects were in the low-normal range, which is known to be associated with above-average fractional elimination rates of endogenous apoA-I (20–25).

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