Early Incorporation of Cell-Derived Cholesterol into Pre-β-Migrating High-Density Lipoprotein†

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ABSTRACT: Cultures of human skin fibroblasts were labeled to high cholesterol specific activity with [^{3}H]cholesterol and incubated briefly (1–3 min) with normal human plasma. The plasma was fractionated by two-dimensional agarose–polyacrylamide gel electrophoresis and the early appearance of cholesterol label among plasma lipoproteins determined. A major part of the label at 1-min incubation was in a pre-β-migrating apo A-I lipoprotein fraction with a molecular weight of ca. 70 000. Label was enriched about 30-fold in this fraction relative to its content of apo A-I (1–2% of total apo A-I). The proportion of label in this lipoprotein was strongly correlated with its concentration in plasma. Further incubation (2 min) in the presence of unlabeled cells demonstrated transfer of label from this fraction to a higher molecular weight pre-β apo A-I species, to low-density lipoprotein, and to the α-migrating apo A-I that made up the bulk (96%) of total apo A-I in plasma. The data suggest that a significant part of cell-derived cholesterol is transferred specifically to a pre-β-migrating lipoprotein A-I species as part of a cholesterol transport transfer sequence in plasma.

Cholesterol from peripheral cell membranes is available for transport into interstitial fluids and plasma. It is transferred over a period of time into all the major plasma lipoprotein fractions. However, the mechanism involved in the incorporation of cell membrane cholesterol into plasma lipoproteins is known only in outline. High-density lipoprotein (HDL), and in particular its major protein, apolipoprotein A-I (apo A-I), appears to play a particularly important role as an acceptor of cell-derived cholesterol, as an acceptor of diffusion-limited efflux (Phillips et al., 1980) or, more directly, as a ligand for a receptor-mediated interaction with the cell surface (Oram et al., 1983; Graham & Oram, 1987). Other recent data implicate a minor subfraction of HDL containing only apo A-I as the most active factor in this reaction (Fielding & Fielding, 1981). However, little is known of the intermediate steps involved in cholesterol transport from the moment of release into the medium to the incorporation of this cholesterol in the major lipoproteins of plasma. In this research, we have followed the incorporation of cholesterol into plasma from cultured fibroblasts that had been labeled to high specific activity. The recovery of very early protein acceptors of this label greatly enriched in cholesterol label among plasma lipoproteins suggests a specific transport chain for cell-derived cholesterol.

Experimental Procedures

Plasma was obtained from the blood of 10 normolipemic volunteers who had fasted overnight. The blood was collected and cooled immediately in ice water either into a final concentration of 0.0025 M sodium citrate (pH 7.4) or else into the mixture of protease inhibitors used by Cardin et al. (1984) modified by the exclusion of disodium ethylenediaminetetraacetate and polybrene. Final concentrations of inhibitors in plasma were 8.5 μg/mL aprotinin, 5 μg/mL D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK), 2 mM benzamidine, 20 μg/mL soybean trypsin inhibitor, and 20 μg/mL lima bean trypsin inhibitor. Blood temperature was <5 °C within 10 s of blood draw. In some experiments, coagulation was inhibited by the inclusion of only the thrombin antagonist PPACK (Kettner & Shaw, 1981). Coagulation under these conditions was inhibited for at least 5 min at 37 °C, without detectable detachment of fibroblasts from a cultured cell monolayer. No difference was found either in the rate of cholesterol efflux or in the pattern of lipoproteins separated by electrophoresis under these different conditions.

Incubation Conditions. Normal skin fibroblasts were cultured at 37 °C in 6-cm plastic dishes in Dulbecco modified Eagle's medium containing 10% fetal calf serum to near confluence. At this point the dishes contained 8–10 μg of cell membrane cholesterol per dish. Some dishes of cells were prelabeled for 48 h with 1–1.5 mCi of [1,2-^{3}H]cholesterol (New England Nuclear, Boston, MA) that had been first complexed with fetal calf serum before incorporation into the culture medium (Fielding & Fielding, 1981). Final specific activity in the cells was 1–2 × 10^8 dpm/μg of cholesterol. To determine whether transfer of label to plasma was a cell-dependent pathway, in some experiments the cell monolayer was substituted with [^{3}H]cholesterol-labeled albumin–agarose covalent complex. The complex was prepared from recrystallized human albumin and CNBr–agarose (Porath et al., 1967). Ice-cooled human plasma, obtained as indicated above, was brought to 37 °C (1–2 min) and used immediately. In individual experiments, the dishes of cells were washed (4 × each) with phosphate-buffered saline (pH 7.4) and then incubated with anticoagulated human plasma for 1–5 min at 37 °C at 1.5 cycles/s on the plate of an orbital mixer (LabLine, Melrose Park, IL). Samples were taken at intervals for determination of medium radioactivity and its distribution among plasma lipoproteins. In some experiments, plasma collected after 1 min from the labeled cells was transferred to a second dish of unlabeled cells, and the further transfer of labeled cholesterol followed as a function of time. Samples of plasma medium were taken at intervals for analysis.

Analysis by Two-Dimensional Electrophoresis. The distribution of lipoprotein fractions in plasma, particularly those of HDL, was determined by two-dimensional agarose–polyacrylamide gel electrophoresis. One-dimensional agarose gel electrophoresis was carried out in 0.75% agarose in 50 mM

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barbital buffer (pH 8.6) on Gelbond (FMC, Rockland, ME) at 0 °C. Native plasma (20 μL) was added to a 1.5-cm sample channel. Electrophoresis was carried out until a marker of albumin stained with bromophenol blue had migrated 7.5 cm.

In the second dimension, polyacrylamide gel electrophoresis was carried out on a 2–15% gradient (14 × 16 cm) on which a 2.5-cm stacking gel was overlaid. Two pieces (0.5 × 7.0 cm) were cut from the agarose gel such that each included one-third of the agarose electrophoresis strip. These were laid on the stacking gel end to end and fixed in place with 0.75% agarose. Each polyacrylamide gel would therefore contain two two-dimensional gel patterns, one of which, developed by transfer and immunoblotting to identify the pattern of apoprotein species, would be used as template for the second gel to detect cholesterol radioactivity.

Electrophoresis was carried out at 0 °C in 0.025 M tris-(hydroxymethyl)aminomethane (Tris)–glycine buffer (pH 8.3) at 300 V for 1 h and then at 400 V for 4 h (a total of 1900 V h). Temperature was monitored throughout and maintained with a Haake A80 recirculating bath.

At the end of the separation the gel was divided in half longitudinally. One half was maintained at 2 °C in foil; the other was used for electrophoretic transfer (TransBlot, Hoefer Scientific, San Francisco, CA) to nitrocellulose membrane (Sartorius, 0.45 μm). Transfer was carried out in 0.025 M Tris–glycine buffer (pH 8.3) containing 20% v/v methanol for 10 h at 30 V.

In experiments to determine the apparent molecular weight of lipoprotein complexes, electrophoresis in the second dimension was carried out to equilibrium (20 h electrophoresis at 200 V) in a linear nondenaturing 3–24% polyacrylamide gradient gel, together with protein molecular weight standards (Pharmacia, Piscataway, NJ).

Identification of Apolipoproteins. Polyclonal antibodies to apolipoproteins A-I, A-2, and B were raised in rabbits, as previously described (Fielding & Fielding, 1981). Nitrocellulose blots were treated with 1% antibody in 5% bovine serum albumin in 25 mM Tris-HCl and 0.15M NaCl at pH 8.0 for 1 h and then with either protein A or goat antirabbit immunoglobulin G (IgG) (Cappel, Malvern, PA) labeled with ^{125}I (Markwell, 1982). For quantitation of antigen on the blot, a range of standards of known apo A-I mass (0.01–1.0 μg) was applied directly to the same nitrocellulose sheet. Radioactivity was detected by autoradiography at −70 °C on XAR-2 Kodak film with an intensifier screen (Cronex, Du Pont, Wilmington DE). Individual labeled membrane areas identified by film were subsequently cut out and counted by use of a Searle γ scintillation counter.

Determination of Lipoprotein Lipids. Identification of lipoproteins on the second half of the two-dimensional gel was carried out by using the autoradiogram developed from the anti apo A-I blot as template. Lipoprotein areas were cut out with a razor blade, extracted with chloroform–methanol (1:1 v/v) for 48–72 h, and then analyzed for cholesterol or phospholipid content. Free and ester cholesterol were determined with cholesterol oxidase in a volume of 100 μL with homovanillic acid (Fielding, 1985). Phospholipid content was determined with 1,6-diphenyl-1,3,5-hexatriene (London & Feigenson, 1978). In both cases, samples were read by using a Spex 1680 fluorolog spectrometer with DM1B coordinator. Lipid standards of cholesterol or egg lecithin over the range 0.01–1.0 μg (about 10^3–10^5 photons) were also determined.

RESULTS

Pre-β-Migrating Apo A-I Species. Plasma was fractionated by the two-dimensional agarose–polyacrylamide gel electrophoresis described above. Following electroblotting, molecular species containing apolipoprotein apo A-I were identified immunologically (Figure 1). Recovery of lipids and proteins from the gel was determined from the sum of apo A-I, and lipids, recovered in the individual fractions identified in Figure 1. Total recovery of apo A-I from the blot (to which the equivalent of 6.67 μL of plasma had been applied) was 96.5 ± 6.5% compared to the apo A-I in plasma. Total recovery of cholesterol with apo A-I [excluding that proportion comigrating with low-density lipoproteins (LDL)] was 86.1 ± 15.4% and recovery of phospholipid was 86.0 ± 10.0% compared to the concentration of these lipids in plasma HDL. These data indicate that apo A-I, cholesterol, and phospholipid are efficiently recovered for analysis from the electrophoresis matrix.

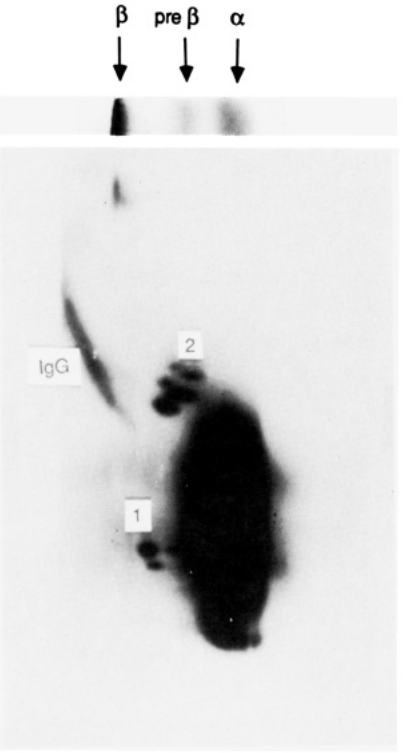

FIGURE 1: Distribution of apo A-I in normal plasma determined by two-dimensional gel electrophoresis. Sequential electrophoresis on agarose and polyacrylamide was carried out as described under Experimental Procedures. Protein in the gel was transferred to nitrocellulose and blotted first with unlabeled rabbit polyclonal anti-apo A-I antibody and then with ^{125}I-labeled protein A. Labeled areas: IgG, immunoglobulin G; 1, LpA-$I_{pre\text{-}\beta 1}$; 2, LpA-$I_{pre\text{-}\beta 2}$. The β-migrating apo A-I comigrates with low-density lipoprotein, as described in the text. The electrophoresis standards of the major plasma lipoprotein classes shown were obtained from the one-dimensional electrophoresis of plasma lipids stained with Sudan Black.

Although it is generally recognized that apo A-I in native plasma is present in multiple distinct molecular species (LpA-I species), no generally accepted nomenclature has been established. Here fractions are characterized on the basis of their migration rate in agarose gel electrophoresis, relative to the major lipoprotein classes (β, pre-β, and α) and in order of migration rate on polyacrylamide gel gradients.

The major part of apo A-I [(94.1 ± 3.1)%, $n = 21$] was represented by a broad band of rapid (α) migration. In addition, there were always present two groups of small punctate-stained species, with pre-β mobility in the first dimension and a migration rate in the second dimension similar to the fastest and slowest components of bulk apo A-I (LpA-$I_{pre\text{-}\beta 1}$ and LpA-$I_{pre\text{-}\beta 2}$). These contained respectively 2.8 ± 2.1% and 2.3 ± 0.8% of apo A-I radioactivity. The former consisted

Table I: Composition of Pre-β-Migrating LpA-I Species[a]

	FC (wt %)	PL (wt %)	apo A-I (wt %)	FC/PL (w/w)	FC/A-I (w/w)	A-I/PL (w/w)
pre-β 1	7.6 ± 2.3	44.6 ± 4.6	47.5 ± 8.0	0.19 ± 0.11	0.16 ± 0.04	1.14 ± 0.44
pre-β 2	5.7 ± 1.0	73.8 ± 2.6	21.5 ± 4.3	0.08 ± 0.01	0.28 ± 0.10	0.29 ± 0.07

[a] FC, free cholesterol; PL, phospholipid; apo A, apolipoprotein A-I. Values (n = 3) were determined on lipoproteins eluted from agarose–polyacrylamide electrophoresis gels. Areas were identified by apo A-I immunoblotting as described under Experimental Procedures; lipids were then eluted and determined spectrophotometrically. Apo A-I in the same areas was determined by quantitative immunoblotting. On the basis of lipoprotein molecular weight of 71 000 for $Lp_{pre\text{-}\beta 1}$ and 325 kDa for $Lp_{pre\text{-}\beta 2}$, determined by equilibrium gradient gel electrophoresis, the predicted molecular composition of these lipoproteins is apo A-I 1.2, FC 13.9, and PL 40.9 for $Lp_{pre\text{-}\beta 1}$ and apo A-I 2.5, FC 47.2, and PL 318 for $Lp_{pre\text{-}\beta 2}$. These values are calculated on the basis of the following molecular weights: apo A-I, 28 331; FC, 386; PL, 775.

Table II: Distribution of [^{3}H]Cholesterol Radioactivity among LpA-I Species

	pre-β 1			pre-β 2			α		
expt	A-I (%)	^{3}H (%)	^{3}H/A-I	A-I (%)	^{3}H (%)	^{3}H/A-I	A-I (%)	^{3}H (%)	^{3}H/A-I
1	2.0	43	21.5	5.2	5	1.0	92.8	39	0.42
2	2.1	77	36.6	1.0	10	10.0	96.9	8	0.08
3	2.5	78	31.2	0.4	11	27.5	97.0	8	0.08
4	1.4	39	28.0	2.9	50	17.2	95.4	8	0.08
5	0.4	12	30.0	1.9	1	0.5	97.7	87	0.89
6	0.3	11	36.6	2.6	24	9.2	97.1	42	0.38
7	1.0	33	33.0	1.0	6	6.0	95.5	42	0.44
8	0.7	18	25.7	4.0	6	1.5	94.3	70	0.74
mean	1.3	39	30.3	2.4	14	9.1	95.8	38	0.39
SD	0.8	27	5.2	1.6	16	9.3	1.6	30	0.29

[a] Values are of the distribution of radioactivity following the incubation for 1 min, 37 °C, of unlabeled plasma with [^{3}H]cholesterol-labeled fibroblasts. In six of eight of these experiments, β-migrating apo A-I comigrating with LDL represented <0.5% of total apo A-I. In two experiments (no. 6, 7) β-migrating apo A-I was 2.6% and 1.3% of total apo A-I and in these experiments was associated with 23% and 17% of total [^{3}H]cholesterol radioactivity, respectively. In the other experiments it represented 0–2% of total counts. Total plasma apo A-I by immunoassay (Ishida & Fielding, 1987) was 1.24 ± 0.04 mg/mL.

of a major and a minor component, with 1.9 ± 0.5% and 0.6 ± 0.3% of total label, respectively, while the latter consisted of three to five components (Figure 1) whose proportions were quite variable between individuals. The former group migrated slightly more slowly during agarose gel electrophoresis (Figure 1). As all these fractions reacted with cell-derived cholesterol in these studies, they were considered together in this study, although they show small differences in charge and apparent molecular weight (Figure 1). A slowly migrating species of β mobility that comigrated with LDL contained 1.2 ± 0.8% of total apo A-I label. The second major protein of HDL (apo A-2) was present only with α-migrating apo A-I (Figure 2). When similar experiments were carried out with antibodies to apolipoproteins B, C-III, D, and E, there was also no significant label comigrating with $LpA\text{-}I_{pre\text{-}\beta 1}$ or $LpA\text{-}I_{pre\text{-}\beta 2}$.

Further characterization of pre-β-migrating apo A-I was carried out in terms of lipid composition and apparent molecular weight (Table I). The $LpA\text{-}I_{pre\text{-}\beta 1}$ fraction contained approximately equal proportions by weight of apo A-I and phospholipid, with a smaller amount of free cholesterol. The $LpA\text{-}I_{pre\text{-}\beta 2}$ fraction contained a much lower ratio of protein to phospholipid. The former had an mean apparent M_r of 71 kDa (Ishida & Fielding, 1987) compared to an average of 325 kDa for the larger fraction. The estimates are based on migration rates in the nondenaturing gradient gel electrophoresis system described under Experimental Procedures.

Transfer of Cellular Cholesterol to Plasma Lipoproteins. When plasma was briefly (1 min) exposed to cultured fibroblasts labeled with [^{3}H]cholesterol, there was a rapid appearance of label in the medium. Total flux was determined as the rate of appearance of label in the medium relative to the specific activity of cholesterol in the cells and was 0.01–0.02 μg/min (five experiments), a value comparable to rates obtained in previous studies under the same conditions over a 60-min assay period (Fielding & Fielding, 1981). Of this total, 91 ± 7% was recovered in the α or pre-β electrophoretic zones, comigrating in two-dimensional electrophoresis with apo A-I. These data provide strong support for the preeminent role of apo A-I in cholesterol transport in normal human plasma.

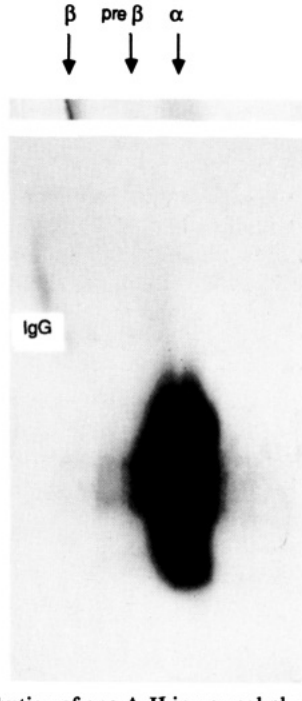

FIGURE 2: Distribution of apo A-II in normal plasma determined by two-dimensional gel electrophoresis. Separation was carried out as described in the legend to Figure 1. No detectable radioactivity is observed comigrating with the β and pre-β fractions of apo A-I.

Among the three zones represented ($LpA\text{-}I_{pre\text{-}\beta 1}$, $LpA\text{-}I_{pre\text{-}\beta 2}$, and $LpA\text{-}I_{\alpha}$), the label was very unevenly distributed (Table II). Although the first fraction contained only a small part of the apo A-I, it contained about half the radioactivity, so that its specific activity (relative to apo A-I) was about 30-fold greater than for plasma as a whole. There was wide variation among plasma samples of the radioactivity recovered in this fraction after 1 min of incubation, but there was a high degree of correlation ($r = 0.95$, $p < 0.01$) between radioactivity and

Table III: Effects of an Unlabeled Plasma Chase on the Distribution of Early [^{3}H]Cholesterol Radioactivity[a]

LpA-I fraction	1-min pulse	1-min pulse + 2-min chase
pre-β 1	38.3 ± 13.1	3.2 ± 3.0, $p < 0.02$
pre-β 2	21.2 ± 16.8	33.3 ± 38.7
α	21.0 ± 16.2	43.5 ± 25.6
β	7.5 ± 8.8	18.2 ± 17.4, $p < 0.05$

[a] Values given are means ± SD (n = 6) of the percentage distribution of total applied radioactivity following 1 min of incubation at 37 °C with [^{3}H]cholesterol-labeled cells, as described in the legend to Table II, followed by further incubation for 2 min at 37 °C with unlabeled cells.

the mass of this fraction present. This would be consistent with the concept that the concentration of this lipoprotein is rate-limiting for the delivery of cell-derived cholesterol to more complex lipoproteins. There was a smaller enrichment of label in LpA-$I_{pre\text{-}\beta 2}$, while the major fraction (95.8 ± 1.6) of HDL (LpA-I_α) contained only about one-third of the radioactivity.

When labeled cholesterol–albumin complex replaced the labeled cell monolayer as donor, there was no selective incorporation of radioactivity into either $Lp_{pre\text{-}\beta 1}$ or $Lp_{pre\text{-}\beta 2}$ relative to the distribution of apo A-I. Indeed, relatively little label was associated with LpA-$I_{pre\text{-}\beta 1}$. Compared to the ratios shown in Table II, the ratios of ^{3}H/apo A-I in LpA-$I_{pre\text{-}\beta 1}$, LpA-$I_{pre\text{-}\beta 2}$, and LpA-I_α were 0.2 ± 0.2, 1.2 ± 1.2, and 1.0 ± 0.1, respectively (means ± standard deviation, three experiments).

The data above indicated that early cholesterol radioactivity from the cell surface (but not from albumin–cholesterol complex) was preferentially incorporated into the pre-β-migrating fractions of apo A-I. Indeed, in the studies in Table II, these fractions, taken together, contained on average nearly 60% of the label, together with less than 4% of the apo A-I. Counts were found in all the pre-β apo A-I species.

No detectable labeled cholesteryl ester was recovered in the pre-β apo A-I fractions over the time scale used in these studies when total labeled radioactivity was fractionated by thin-layer chromatography.

Two explanations of these data seemed feasible. There might be several different pathways by which early radioactivity entered HDL fractions. Alternatively, the major part might enter via one pathway, and the difference in distribution seen at 1 min of incubation with the labeled cells might represent differences in the rate of transfer of label from this species and secondary or later acceptors of cholesterol label. To obtain further information on this possibility, plasma that had been labeled for 1 min at 37 °C was transferred to an equivalent dish of unlabeled cells for 2 min under the same conditions, such that the early label on these lipoproteins would be replaced by unlabeled sterol. If a specific major pathway was present in plasma, there should be a significant decrease in specific radioactivity in that fraction during the 2-min chase.

As shown in Figure 3, when plasma that was highly labeled in the LpA-$I_{pre\text{-}\beta 1}$ zone was further incubated with unlabeled cell cholesterol, there was a rapid and essentially quantitative loss of label in that fraction, and this was recovered in LpA-$I_{pre\text{-}\beta 2}$. When these experiments were carried out over the range of initial label concentrations found in all experiments, a similar loss of label from LpA-$I_{pre\text{-}\beta 1}$ was evident (Table III). More than 90% of label was lost from this species within 2 min, such that at the end of the period there was little or no enrichment of this lipoprotein zone over total plasma apo A-I. There was on average a 2.5-fold increase in the label present in the β-migrating zone and smaller increases in the proportion of label in the other fractions.

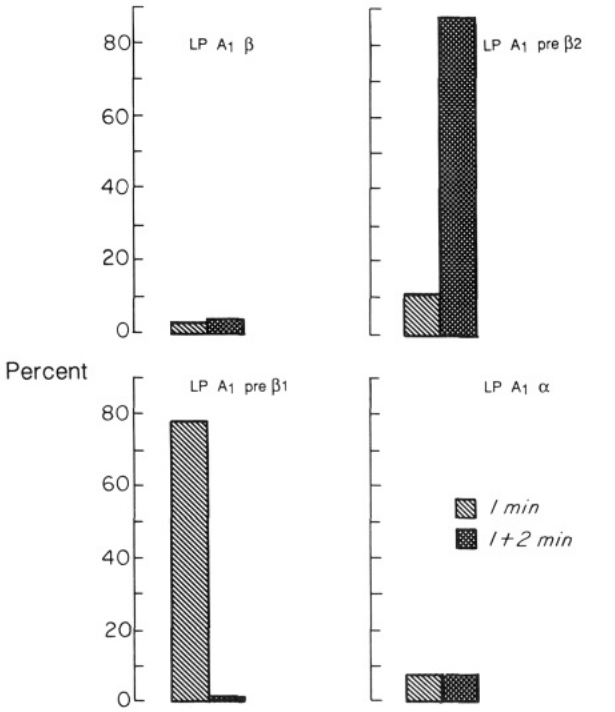

FIGURE 3: Distribution of apo A-I- associated radioactivity following the incubation of unlabeled plasma with [^{3}H]cholesterol-labeled fibroblasts for 1 min at 37 °C followed by a further 2 min of incubation with unlabeled cells. The vertical axis represents the proportion of total radioactivity recovered under these conditions in the apo A-I fractions illustrated in Figure 1.

These data suggest that the major part of early cholesterol label derived from cell membranes is transferred to the minor lipoprotein species LpA-$I_{pre\text{-}\beta 1}$. It is likely that a major part of this label is subsequently transferred to the LpA-$I_{pre\text{-}\beta 2}$ fraction, although this was most clearly evident when the proportion of label in the precursor fraction was large.

Discussion

Previous studies using immunoaffinity chromatography suggested a key role for lipoproteins containing apo A-I (and, in particular, apo A-I species not containing apo A-2) as acceptors of cell-derived cholesterol (Fielding & Fielding, 1981). The use of high cholesterol specific activities and very brief incubation periods in the present study has allowed more specific identification of the early lipoprotein acceptors of cell-derived labeled cholesterol.

When unfractionated plasma was incubated for 1 min at 37 °C with [^{3}H]cholesterol-labeled fibroblasts, a major fraction of the radioactivity recovered comigrated with the pre-β-migrating minor lipoprotein fraction $Lp_{pre\text{-}\beta 1}$. This was always highly enriched in label (relative to apo A-I) compared to the major part of HDL. The composition of this fraction is similar to that recently reported for a small component of synthetic recombinant HDL obtained by the dialysis of apo A-I, egg lecithin, and cholesterol from cholate solution (Nichols et al., 1987). However, this particle, further purified by centrifugation, was suggested on the basis of cross-linking studies to contain two apo A-I molecules, rather than one as suggested from this study. This would lead from compositional data to a calculated M_r of 123 kDa, about twice that found here (71 kDa). Further studies may indicate that the native and synthetic recombinant lipoproteins are monomer and dimer of similar structures. The apparent molecular weight of the plasma lipoprotein was the same as that of the pre-β apo A-I electrophoretic fraction observed earlier (Ishida & Fielding,

1987). In that study, the lower molecular weight forms of pre-β apo A-I were selectively seen, probably owing to the shorter electrophoretic transfer (1.5 h vs 10 h in this study) or, perhaps, their greater concentration or stability. A pre-β-migrating lipoprotein has also been described by Kunitake et al. (1985) with a molecular weight of 80 000. This fraction had 91 ± 3% protein by weight, a different composition from the synthetic recombinant of Nichols et al. (1987) or the lipoprotein described here. Its concentration in plasma (on average 14.4% of total apo A-I) was also higher than that found in this study. It may represent a product of one of the species observed in this study.

Enrichment of label was also observed, although to a lesser extent, in a second fraction, also with pre-β electrophoretic migration (LpA-$I_{pre\text{-}\beta 2}$). This fraction has a composition similar to that of the larger, discoidal recombinant obtained from synthetic mixtures of apo A-I, phospholipid, and cholesterol (Matz & Jonas, 1982; Nichols et al., 1987). The apparent molecular weight of LpA-$I_{pre\text{-}\beta 2}$ by gradient gel electrophoresis was 325 000, similar to that calculated for the larger synthetic recombinant from cross-linking and compositional data (about 287 000). Because further incubation of plasma clearly demonstrated the transfer of label from the first to the second lipoprotein when the proportion of the former was sufficiently high, it seems likely that when label is seen initially in LpA-$I_{pre\text{-}\beta 2}$, this represents cholesterol derived in the very early part of incubation from LpA-$I_{pre\text{-}\beta 1}$ and that a precursor–product relationship exists between these particles. However, some direct labeling of LpA-$I_{pre\text{-}\beta 2}$ and LpA-I_{α} cannot be excluded.

The specific early transfer of cholesterol from cell membranes clearly evident in these studies might seem anomalous in view of the randomness of diffusion (Phillips et al., 1980) or the general reactivity of HDL suggested from studies of its receptor function (Oram et al., 1983). However, several potential mechanisms could explain the selectivity of early labeling that has been observed in these studies. The small size of the early acceptors of cholesterol may favor the insertion of cholesterol (McLean & Phillips, 1981). Their small size and, presumably, greater diffusion coefficient would accelerate their entry into the unstirred water layer surrounding the cells or to receptor sites on the membrane surface, at rates greater than those of larger lipoproteins. Finally, phospholipid composition could also play a role in the selective binding of cholesterol (Lund-Katz et al., 1986).

The mechanism of the transfer observed involving LpA-$I_{pre\text{-}\beta 1}$ is of interest. Since early cholesterol label was effectively transferred from the low to the high molecular weight forms of pre-β Lp A-I, rather than diluted with the bulk of plasma or even HDL cholesterol, the movement of label observed in these experiments could not be the result of simple diffusion: either a highly specific transfer of cholesterol between these lipoproteins must take place or else the smaller pre-β HDL fraction must be converted to the larger, along with its cholesterol content, during the metabolism of cholesterol in plasma.

Two pieces of evidence seem to support the latter interpretation. First, the composition of the larger pre-β particle is consistent with that of a discoidal synthetic recombinant of apo A-I and lipids previously proposed as a fusion product of small subunits, with the generation of free apo A-I (Nichols et al., 1987). Second, the content of cholesterol per particle is not increased in proportion to particle apparent molecular weight. The data shown in Table I seem most consistent with the hypothesis that LpA-$I_{pre\text{-}\beta 2}$ could be formed by the fusion of four of the smaller particles, with the release of free apo A-I and, probably, the further gain of phospholipid, concomitant with the formation of a discoidal particle. An HDL transforming factor has been identified in plasma, which catalyzes the conversion of small HDL into larger HDL and free apo A-I (Rye & Barter, 1986). It seems an attractive possibility that the functions of such a fraction might include catalysis of the early metabolism of cell-derived cholesterol.

In any event, this study of cholesterol transport in normal plasma provides evidence for the early labeling of a minor apo A-I containing lipoprotein. This label subsequently appears, at least in part, in a particle of greater apparent molecular weight, which may be a fusion product, before transfer to the major plasma lipoproteins. This indicates a high degree of structural specificity in the early steps of cholesterol transport from cell membranes to plasma lipoproteins.

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