Lipoprotein remodeling generates lipid-poor apolipoprotein A-I particles in human interstitial fluid

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1Magdalen College, University of Oxford, Oxford, United Kingdom; 2Department of Surgical Research and Transplantology, Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; 3Department of Advanced Medical Technology and Development, BML, Kawagoe, Japan; 4Oxford PharmAssist, London, United Kingdom; 5Department of Virology II, National Institute of Infectious Disease, Tokyo, Japan; and 6Cardiovascular Genetics Unit, University of Utah School of Medicine, Salt Lake City, Utah

Submitted 25 June 2012; accepted in final form 5 December 2012

Miller NE, Olszewski WL, Hattori H, Miller IP, Kujirako T, Oka T, Iwasaki T, Nanjee MN. Lipoprotein remodeling generates lipid-poor apolipoprotein A-I particles in human interstitial fluid. Am J Physiol Endocrinol Metab 304: E321–E328, 2013. First published December 11, 2012; doi:10.1152/ajpendo.00324.2012.—Although much is known about the remodeling of high density lipoproteins (HDLs) in blood, there is no information on that in interstitial fluid, where it might have a major impact on the transport of cholesterol from cells. We incubated plasma and afferent (prenodal) peripheral lymph from 10 healthy men at 37°C in vitro and followed the changes in HDL subclasses by nondenaturing two-dimensional crossed immunoelectrophoresis and size-exclusion chromatography. In plasma, there was always initially a net conversion of small pre-β-HDLs to cholesteryl ester (CE)-rich α-HDLs. As a result, in lymph, there was only net production of pre-β-HDLs from α-HDLs. Endogenous cholesterol esterification rate, cholesteryl ester transfer protein (CETP) concentration, CE transfer activity, phospholipid transfer protein (PLTP) concentration, and phospholipid transfer activity in lymph averaged 5.0, 10.4, 8.2, 25.0, and 82.0% of those in plasma, respectively (all P < 0.02). Lymph PLTP concentration, but not phospholipid transfer activity, was positively correlated with that in plasma (r = +0.63, P = 0.05). Mean PLTP-specific activity was 3.5-fold greater in lymph, reflecting a greater proportion of the high-activity form of PLTP. These findings suggest that cholesterol esterification rate and PLTP specific activity are differentially regulated in the two matrices in accordance with the requirements of reverse cholesterol transport, generating lipid-poor pre-β-HDLs in the extracellular matrix for cholesterol uptake from neighboring cells and converting pre-β-HDLs to α-HDLs in plasma for the delivery of cell-derived CEs to the liver.

Apolipoprotein A-I; cholesterol; high-density lipoproteins; interstitial fluid; peripheral lymph

HIGH DENSITY LIPOPROTEINS (HDLs) are a heterogeneous group of protein-lipid complexes that in humans have a hydrated density >1.063 g/ml and apolipoprotein (apo)A-I as the principal protein component. Different subclasses differ in density, size, and charge, reflecting differences in composition. Originally postulated as having the single function of transporting cholesterol from peripheral cells to the liver (15), now referred to as reverse cholesterol transport, several other properties of HDLs have since been identified (5, 50).

Glomset’s concept (15) of reverse cholesterol transport, based on experiments with plasma HDLs and erythrocytes, was that conversion of unesterified cholesterol (UC) to cholesteryl ester (CE) in HDLs by lecithin-cholesterol acyltransferase (LCAT) generates a chemical potential gradient of UC between the particles and the surface membranes of cells. It is now clear that, while this mechanism operates when cells are exposed to plasma, it probably does not contribute to the efflux of UC from most cells in vivo. First, the interstitial fluid that occupies the interstices of the extracellular matrix contains very little cholesterol-esterifying activity (47, 54). Second, the HDLs in interstitial fluid are enriched in UC (36, 54), indicating that the rate at which they acquire cell-derived UC exceeds its subsequent rate of esterification. In tissue culture, transfer of UC from cells is not dependent on LCAT but occurs during the binding of small lipid-poor apoA-I containing HDLs with pre-β-electrophoretic mobility (pre-β-HDLs) to the ABCA1 transporters of cell membranes (6, 45).

In human plasma, pre-β-HDLs account for only about 5% of total apoA-I (6, 33). When plasma is incubated at 37°C in vitro, pre-β-HDLs decrease at first and may disappear because of conversion to CE-rich α-HDLs, a process dependent on LCAT (18, 39). During continued incubation, pre-β-HDL concentration recovers, rising to values that after 24 h may exceed the preincubation value (20). The mechanism of pre-β-HDL production involves the fusion or destabilization of α-HDLs (23) and is catalyzed by the phospholipid transfer (PLTP) and cholesteryl ester transfer (CETP) proteins (28, 51) that circulate in plasma mostly in association with HDLs (8, 29). To date, all studies of HDL remodeling in vitro have used plasma or HDLs isolated from plasma. The results have provided important insights into the mechanisms of lipid transport between organs and tissues. However, there is no information on HDL remodeling in interstitial fluid, where it might have a major impact on the movement of lipids out of cells.

Interstitial fluid is formed by the transfer of water, electrolytes, and macromolecules of plasma through the pores of capillary endothelium (27). Water and electrolytes move under hydrostatic pressure and macromolecules by convection (27). The majority of water and electrolytes return to plasma by diffusion at the venous end of capillaries, the residuum permeating the extracellular matrix before entering lymphatic capillaries (43). The return of macromolecules to blood is partitioned between these two routes, the predominant one for a given molecule being dictated by its size (31). Because lipoproteins are expected to exit entirely via the lymphatic route...
We have studied the remodeling of HDLs when afferent peripheral lymph from healthy humans was incubated at 37°C and compared it with that in plasma from the same subjects. To explore the mechanisms underlying observed differences, measurements were also made of cholesterol esterification rate and of the concentrations and activities of PLTP and CETP. The results suggest that interstitial fluid may be a major site of production of pre-β-HDLs, consequent upon a near-zero cholesterol esterification rate and much greater specific activity of PLTP than in plasma.

METHODS

Clinical Procedures

Ten healthy male subjects were studied (Table 1). All had been screened for cardiovascular, renal, hepatic, and endocrine disorders as previously described (35). Blood samples were also examined for recreational drugs. The study had been approved by the appropriate institutional review boards, and the subjects gave informed written consent.

Lymph vessel cannulations were performed under sterile conditions. The subjects were fasted for 12–14 h beforehand but were allowed fat-free drinks. The procedure has been described (35). Briefly, an area of skin 6–10 cm above the ankle was anesthetized and an incision 15–20 mm wide made in the center. Under an operating microscope, a suitable subcutaneous lymph vessel was identified. A second smaller incision was made above the first, through which a tapered sterile siliconized polyethylene cannula was passed. The vessel was opened, and the first distal valve was destroyed. The cannula was passed into the vessel and secured with a ligature, and the other end opened, and the first distal valve was destroyed. The cannula was passed through the cap of a plastic vial. For some assays, the vial contained no additives; for others it contained 2 mg disodium EDTA powder. The vial was kept in crushed ice, contained in an expanded polystyrene holder strapped to the leg (10). The volunteer then returned to the metabolic ward, where he remained ambulant and consumed a eucaloric solid food diet [fat-protein-carbohydrate (%calories), 21:20:79; total fat, 65 g/day; saturated-polyunsaturated-mono-unsaturated fatty acids (%weight), 44:23:33; cholesterol, 186 mg/day]. Alcohol was not permitted. The collection tubes were changed every two or three hours and centrifuged at 4°C. Venous blood (10 ml) was collected during each 2- to 3-h collection period and treated identically to the lymph samples. All samples were stored at −80°C before analysis. Shipments from the United Kingdom to Japan for PLTP and CETP assays were done on dry ice by a courier.

Laboratory Procedures

Lipid, apoA-I, and protein concentrations. All assays were performed in duplicate with intra-assay coefficients of variation (CV) <2%. Total cholesterol (TC), triglycerides (TG), and phospholipid (PL) were quantified using microbial enzymes (Sigma-Aldrich) and the colorimetric Trinder reaction; CE was calculated as TC minus UC. apoA-I was quantified by radioimmunoassay, using a goat polyclonal antibody against delipidated human apoA-I (International Immunology) in the presence of detergent (TWEEN 20, 2.5% vol/vol) and accelerator (polyethylene glycol 6000, 3% wt/vol). Haptoglobin (300 kDa), C-reactive protein (120 kDa), albumin (67 kDa), and α1-acid glycoprotein (44 kDa) were assayed by Laurell rocket immunoelectrophoresis (23, 25).

Concentrations and activities of lipid-transfer proteins. CETP concentration was quantified by a sandwich enzyme-linked immunosorbent assay (ELISA), using two mouse monoclonal antibodies to human CETP (32). CE transfer activity was measured as the rate of transfer of [1,2,6,7-3H]cholesteryl oleate from PL liposomes to low density lipoproteins (LDL) (22). PLTP concentration was quantified by a sandwich ELISA, using two monoclonal antibodies against recombinant human PLTP (41). PL transfer activity was assayed using a 1-palmitoyl-2-[1-14C]palmitoyl phosphatidylcholine-labeled liposome donor/HDL acceptor system (41) in which PL transfer rate is not influenced by CETP. The intra-assay CVs for all assays were <6%.

Distribution of PLTP mass and PL transfer activity by size-exclusion chromatography. Plasma and lymph (1.5 ml) were chromatographed at 0.25 ml/min through tandem Superose-6 HR10/30 columns (Amersham Pharmacia Biotech) and eluted with a buffer containing 10 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4. After discarding the void volume, 0.5-ml fractions were assayed for TC, PLTP concentration, and PL transfer activity (25, 42).

Distribution of apoA-I and lipids by size-exclusion chromatography. Samples of plasma (diluted 3-fold in saline) or lymph (undiluted) were chromatographed at 0.7 ml/min through a Superdex-200 column in series with a Superdex-75 column (both HR10/30; GE Healthcare), using an eluant containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% (wt/vol) Na2EDTA, and 0.1% (wt/vol) sodium azide (33). After the void volume was discarded, 0.2-ml fractions were assayed for UC and TC, or for apoA-I. Recoveries exceeded 90%.

Particle size distribution of PLTP by nondenaturing polyacrylamide gradient gel electrophoresis and Western blotting. Size separation by nondenaturing polyacrylamide gradient gel electrophoresis (ND-PAGGE) was performed in a 5–20% gradient polyacrylamide gel (ATTO) (25, 42). After electrophoresis, gel slices were cut and subjected to gel slicing, which led to detection by immunoblotting.

Table 1. Clinical details of the subjects

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Lymph Flow, ml/h</th>
<th>Chol, mmol/l</th>
<th>Trig, mmol/l</th>
<th>HDL Chol, mmol/l</th>
<th>apoA-I, mg/ml</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>61.7</td>
<td>20.0</td>
<td>0.41</td>
<td>4.6</td>
<td>1.41</td>
<td>1.53</td>
<td>1.01</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>72.9</td>
<td>25.0</td>
<td>2.04</td>
<td>3.5</td>
<td>1.80</td>
<td>1.26</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>97.2</td>
<td>27.2</td>
<td>0.42</td>
<td>3.9</td>
<td>0.75</td>
<td>1.53</td>
<td>1.19</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>60.4</td>
<td>19.3</td>
<td>0.28</td>
<td>3.9</td>
<td>1.50</td>
<td>1.09</td>
<td>0.77</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>70.2</td>
<td>21.7</td>
<td>0.59</td>
<td>3.7</td>
<td>0.95</td>
<td>1.02</td>
<td>0.86</td>
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<td>6</td>
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<td>73.7</td>
<td>25.3</td>
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<td>7</td>
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<td>0.56</td>
<td>4.1</td>
<td>0.53</td>
<td>1.61</td>
<td>1.29</td>
</tr>
<tr>
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<td>22</td>
<td>66.0</td>
<td>21.3</td>
<td>0.63</td>
<td>4.4</td>
<td>0.97</td>
<td>0.71</td>
<td>0.87</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>66.6</td>
<td>21.1</td>
<td>0.71</td>
<td>4.0</td>
<td>1.29</td>
<td>1.33</td>
<td>0.97</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>84.4</td>
<td>26.5</td>
<td>0.50</td>
<td>3.7</td>
<td>1.11</td>
<td>0.97</td>
<td>0.99</td>
</tr>
</tbody>
</table>

BMI, body mass index; Chol, plasma cholesterol; Trig, plasma triglyceride; HDL Chol, plasma high-density lipoprotein (HDL) cholesterol; apoA-I, plasma apolipoprotein A-I. Concentrations of nonlipoprotein proteins (means ± SE in g/l) were as follows: haptoglobin, plasma 0.99 ± 0.19, lymph 0.14 ± 0.02 (lymph-to-plasma ratio 0.14); C-reactive protein, plasma 0.35 ± 0.07, lymph 0.07 ± 0.02 (ratio 0.21); albumin, plasma 42.4 ± 0.5, lymph 14.3 ± 1.1 (ratio 0.34); α1-acid glycoprotein, plasma 0.98 ± 0.12, lymph 0.38 ± 0.06 (ratio 0.39).
no change in pre-incubation was stopped at 24 h. By contrast, in lymph, little or baseline. Thereafter, the concentration increased, until the apoA-I by 2D-CIEP, was always much lower at 2 h than at
apoA-I concentrations in plasma were 6.5 ± 0.4 mg/dl (not significantly different from 0 h), and 2.3 ± 0.6 mg/dl (P < 0.001 relative to 0 h) at 0, 2 and 24 h, respectively. Results qualitatively similar to those observed with normal lymph were seen when plasma from a patient with familial LCAT deficiency was incubated (0, 2, and 24 h: 5.8, 7.3, and 15.9 mg/dl).

Separation by size. A similar difference between plasma and lymph was seen when HDL subclasses were separated by size-exclusion chromatography, using a method that separates the small lipid-poor pre-β-HDLs discretely from the larger CE-rich α-HDLs (33). As shown in Fig. 2, during incubation plasma small pre-β-HDLs decreased substantially during the first few hours, in this case disappearing completely by 2 h and not reappearing until after 8 h. By contrast, in the lymph samples, there was no significant early reduction in pre-β-HDLs, only an increase that was first detectable after 2 h.

Plasma and lymph also differed in the changes that occurred in the distribution profile of apoA-I among α-HDLs. As previously reported (36), before incubation, this was broader in lymph than in plasma. During incubation of lymph, the preponderance of the larger α-HDLs increased relative to the smaller ones, whereas in plasma a general increase in size was observed (Fig. 2).

A shift from smaller to larger HDLs in the distribution of TC was more marked in plasma than in lymph (Fig. 3). In plasma HDLs but not lymph HDLs this was accompanied by an increase in the ratio of CE to UC. In the non-HDL fractions (essentially all LDLs) of plasma UC decreased and CE increased, whereas in the corresponding fractions of lymph no changes in CE or UC were observed.

RESULTS

All subjects had normal plasma concentrations of cholesterol, TG, HDL cholesterol, and apoA-I (Table 1). Lymph-to-plasma ratios of nonlipoprotein proteins of differing molecular weight (see legend for Table 1) were similar to those previously reported in normal humans (31, 35).

Changes in HDL Subclasses During Incubation In Vitro

Separation by charge. Results of a representative experiment are shown in Fig. 1. When plasma was incubated, the concentration of pre-β-HDLs, quantified as pre-β-migrating apoA-I by 2D-CIEP, was always much lower at 2 h than at baseline. Thereafter, the concentration increased, until the incubation was stopped at 24 h. By contrast, in lymph, little or no change in pre-β apoA-I concentration was detected during the first 2 h of incubation, after which it progressively increased. The same pattern was recorded with all plasma-lymph pairs. In samples from six subjects in a single experiment, pre-β apoA-I concentrations in plasma were 6.5 ± 0.6 (mean ± SE) mg/dl at 0 h, 3.4 ± 0.5 mg/dl at 2 h (P < 0.001 relative to 0 h), and 10.9 ± 1.7 mg/dl at 24 h (P < 0.04 relative to 0 h). Concentrations in lymph from the same subjects were 1.5 ± 0.4 mg/dl, 1.4 ± 0.4 mg/dl (not significantly different from 0 h), and 2.3 ± 0.6 mg/dl (P < 0.001 relative to 0 h) at 0, 2 and 24 h, respectively. Results qualitatively similar to those observed with normal lymph were seen when plasma from a patient with familial LCAT deficiency was incubated (0, 2, and 24 h: 5.8, 7.3, and 15.9 mg/dl).

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PLTP Concentration

The concentration of PLTP in lymph averaged 25% of that in plasma ($P < 0.0001$) (Table 2). The correlation coefficient between the two concentrations was $+0.63$, $P = 0.051$. There was no significant difference between plasma and lymph in the PLTP-to-apoA-I ratio.

PL Transfer Activity

Despite the large difference in PLTP mass, the PL transfer activity of lymph was only 18% lower than that of plasma (Table 2), corresponding to a 3.5-fold greater mean PLTP specific activity in lymph. The PL transfer activity of lymph was not correlated with that of plasma ($r = −0.05$).

PLTP Mass and PL Transfer Activity in Lipoprotein Subclasses

The distribution of PLTP mass among lipoprotein subclasses separated by size exclusion is shown in Fig. 4. In plasma, it eluted mostly with small LDLs and large- to medium-sized HDLs. In lymph it eluted with large-sized and medium-sized HDLs, with there being few LDLs. In both matrices, the major peak of PL transfer activity corresponded to small-sized and medium-sized HDLs. Several fractions in the large to medium HDL size range had little or no activity despite measurable PLTP mass. Conversely, several fractions in the small HDL size range and smaller (no detectable cholesterol) had varying degrees of PL transfer activity despite immeasurable PLTP protein. These fractions of very high specific activity were more prevalent in lymph than in plasma.

When the distribution of PLTP protein among fractions separated by ND-PAGGE was examined, it was shifted toward smaller particles in lymph relative to plasma (Fig. 5).

CETP Concentration

CETP concentration in lymph averaged 10% ($P < 0.0001$) of that in plasma (Table 3). There was no significant correlation between the two. The mean CETP-to-apoA-I concentra-
tion ratio in lymph was only about one-half that in plasma ($P < 0.0001$).

**CE Transfer Activity**

CE transfer activity in lymph averaged about 8% of that in plasma ($P < 0.0001$). The specific activity of CETP, calculated as the ratio of CE transfer activity to CETP concentration, was 18% lower in lymph than in plasma ($P < 0.0001$).

**Endogenous Cholesterol Esterification Rate**

The rate of esterification of cholesterol in lymph during a 2-h incubation ($2.99 \pm 1.08 \text{ mmol CE formed·ml}^{-1}·\text{h}^{-1}$, mean ± SD; range $1.27–4.60$) averaged 5% of that in plasma ($59.5 \pm 14.9; 37.6–83.3$) ($P < 0.001$). Lymph-to-plasma ratios of esterification rate averaged $0.051 \pm 0.020$ (range, $0.026–0.100$).

**DISCUSSION**

When we incubated normal plasma, the first change was always a reduction of pre-β-HDL concentration, sometimes leading to the disappearance of the particles. Similar findings have been reported by others and shown to be dependent on the LCAT reaction (20, 39), explaining our failure to observe a decrease in pre-β-HDLs in plasma from a subject with LCAT deficiency. As also reported by others (20, 39), beyond two hours of incubation pre-β-HDL concentration recovered in normal plasma. This second phase has been shown to be catalyzed by PLTP and CETP (51, 58). In contrast to plasma, we observed no reduction of pre-β-HDLs during incubation of any of the 10 lymph samples. We saw only a progressive increase after a brief lag period, the latter presumably attributable to the time taken to reach 37°C, combined with the limited sensitivity.

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**Table 2. Phospholipid transfer activities and phospholipid transfer protein concentrations in plasma and lymph**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>PL Transfer Activity, μmol·ml$^{-1}·h^{-1}$</th>
<th>PLTP Concentration, μg/ml</th>
<th>PLTP Concentration/ apoA-I Concentration</th>
<th>PLTP Specific Activity, μmol·μg$^{-1}·h^{-1}$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Plasma Lymph L/P</td>
<td>Plasma Lymph L/P</td>
<td>Plasma Lymph L/P</td>
<td>Plasma Lymph L/P</td>
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<td>1</td>
<td>6.5 5.6 0.86</td>
<td>17.5 3.7 0.21</td>
<td>17.2 15.3 0.21</td>
<td>0.37 1.51 4.07</td>
</tr>
<tr>
<td>2</td>
<td>4.6 4.7 1.02</td>
<td>9.1 2.0 0.22</td>
<td>10.7 10.5 0.51</td>
<td>2.35 4.65</td>
</tr>
<tr>
<td>3</td>
<td>5.6 6.0 1.07</td>
<td>16.6 5.4 0.33</td>
<td>14.0 16.9 0.34</td>
<td>1.11 3.29</td>
</tr>
<tr>
<td>4</td>
<td>6.8 5.2 0.76</td>
<td>12.0 1.9 0.16</td>
<td>15.7 15.2 0.57</td>
<td>2.74 4.83</td>
</tr>
<tr>
<td>5</td>
<td>6.6 4.6 0.70</td>
<td>14.3 2.7 0.19</td>
<td>16.6 16.0 0.46</td>
<td>1.70 3.69</td>
</tr>
<tr>
<td>6</td>
<td>6.4 7.1 1.11</td>
<td>12.6 4.4 0.35</td>
<td>15.3 23.7 0.51</td>
<td>1.61 3.18</td>
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<td>7</td>
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<td>1.60 3.06</td>
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<tr>
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<td>2.00 3.08</td>
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<td>10</td>
<td>7.3 4.6 0.63</td>
<td>14.5 2.7 0.19</td>
<td>14.7 15.5 0.50</td>
<td>1.70 3.38</td>
</tr>
<tr>
<td>Mean</td>
<td>6.7 5.3 0.82</td>
<td>12.9 3.1 0.25</td>
<td>13.4 15.0 0.56</td>
<td>1.83 3.49</td>
</tr>
<tr>
<td>SD</td>
<td>1.1 0.8 0.19</td>
<td>3.4 1.1 0.07</td>
<td>3.1 4.4 0.24</td>
<td>0.46 0.91</td>
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</table>

*Ratio of phospholipid transfer activity to PLTP concentration. $P$ values were obtained by paired $t$-test. NS, not significant.
of the assays. The associated changes in subclasses of α-HDLs in both matrices were consistent with particle fusion (23), although other explanations are possible. This is the first time that the remodeling of any lipoprotein has been studied in the interstitial fluid of any species.

Our previous studies of apoA-I kinetics in vivo suggested that HDLs remain in the extracellular matrix of peripheral tissues for an average of 29 h before entering blood via lymph (17). Because this exceeds the maximum incubation period used in our present experiments, it suggests that our findings in vitro might also apply in vivo. Thus, although plasma provides a metabolic environment that catalyzes net conversion of pre-β-HDLs to α-HDLs, interstitial fluid may support the reverse process: net production of pre-β-HDLs from α-HDLs.

Given that pre-β-HDLs are the principal acceptors of UC from peripheral cells in direct contact with plasma, such as hepatocytes and vascular endothelium. We have reported that the concentration of apoA-I in peripheral lymph averaged 27 mg/dl in 37 healthy men whose plasma apoA-I concentrations averaged 127 mg/dl (35). If interstitial fluid volume averaged 225 ml/kg (30) and plasma volume 45 ml/kg (48), the extravascular pool of apoA-I in these subjects will have been as large as the plasma pool (about 4 g). Therefore, our findings suggest that interstitial fluid may be a major site of whole body pre-β-HDL production in vivo.

Brunham et al. (4) have presented evidence that in mice the HDLs in mesenteric lymph are formed by the transfer of UC (and presumably also PL) to plasma-derived apoA-I via the ABCA1 transporters of enterocytes. This is considered to be the likely source of the discoidal HDLs devoid of core lipids that are characteristic of mesenteric lymph (16). We have shown that discoidal HDLs are also present in normal human affenter peripheral lymph (36). Further studies in tissue culture will be needed to test the hypothesis that these HDLs are products of the uptake of cellular lipids by pre-β-HDLs generated during the remodeling of α-HDLs in interstitial fluid.
are also pertinent. Although the PLTP concentration of lymph averaged only 25% of that of plasma, its PL transfer activity averaged 80%, corresponding to a 3.5-fold greater PLTP-specific activity in lymph. In plasma, PLTP is known to exist in high-activity and low-activity forms, the former being limited to small- and medium-size HDLs and the latter to large HDLs and small LDLs (11, 42). Our present findings show that the higher PLTP specific activity in lymph is the result of a greater proportion of the high-activity form. The underlying mechanism is open to conjecture. It might be partly because of the fact that high-activity PLTP tends to be associated with smaller lipoproteins, which will cross capillary endothelium more readily than larger ones. Our size-exclusion separations showed that in lymph PLTP was restricted to large-sized and medium-sized HDLs. ND-PAGGE also showed that, although PLTP was present in both LDLs and HDLs in plasma, it was limited to HDLs in lymph. However, preferential transendothelial transfer of smaller particles might not be the entire explanation. Some apolipoproteins have been found to activate the low-activity form of PLTP in vitro (21, 49), and several peripheral cell types have been shown to synthesize and secrete PLTP (12, 40, 49).

Studies in vitro have shown that CETP can also catalyze the remodeling of α-HDLs in plasma to release small lipid-poor apoA-I-containing particles (51, 52). However, in contrast to PLTP, we found no evidence for a greater CETP specific activity in lymph, the CE transfer activity of which was only 8% of that in plasma. Our finding that the lymph-to-plasma ratio of CETP was lower than that of PLTP might be due to the fact that, although in plasma CETP is associated mostly with HDLs, some is bound to larger lipoproteins (9, 11, 57) that do not cross endothelium readily.

Thus, our findings raise the possibility that the interstitial fluid of peripheral tissues generates pre-β-HDL particles, the primary acceptors of cell-derived cholesterol, because of a combination of a very low cholesterol esterification rate and a high ratio of high-activity to low-activity PLTP. This is contrary to the situation in plasma, where a high rate of cholesterol esterification maintains a net conversion of pre-β-HDLs to α-HDLs. However, caution must be exercised when extrapolating from cell-free systems in vitro, and further studies will be needed in cell culture and other experimental systems to confirm that this is also the case in vivo.

GRANTS

N. E. Miller’s laboratory was supported by the British Heart Foundation, Wellcome Trust, Garfield Weston Foundation, Short-Strryker Foundation, European Commission, and Royal Society.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


44. Reichl DJ, Myant NE, Budra DN, Flug JJ. Evidence for the presence of tissue-free cholesterol in low density and high density lipoproteins of human peripheral lymph. Atherosclerosis 37: 489–495, 1980.


