Lipoprotein composition of human suction-blister interstitial fluid[1]

B. Vessby,* S. Gustafson, M. J. Chapman,† K. Hellsing,†† and H. Lithell***

Department of Geriatrics,* Uppsala University, POB 12042, S-750 12 Uppsala, Sweden; Department of Medical and Physiological Chemistry,** Uppsala University, POB 575, S-751 23 Uppsala, Sweden; Groupe de Recherches INSERM sur les Lipoprotéines,† Paris, France; and Clinical Chemistry,†† Uppsala University, Academic Hospital, S-751 85 Uppsala, Sweden

Abstract Interstitial fluid (IF) was obtained in 27 apparently healthy subjects (12 males, 15 females) by applying mild suction (200–250 mm Hg) on the skin either on the midvolar forearm or on the paraumbilical region of the abdomen. The IF concentrations of lipids and apolipoproteins (apo) were studied and compared with those of serum (S). The mean ratio between interstitial fluid and serum (IF/S ratio) varied from 0.14 for forearm apoE to 0.29 for apoA-II on the abdomen. This ratio was consistently lower for apoE, C-II, C-III, and B than for apoA-I and A-II, and significantly lower on the arm than on the abdomen for all apolipoproteins studied. The IF/S ratios showed marked variations among individuals. However, interstitial fluid apolipoprotein concentrations at different blister sites were highly correlated within each individual. Studies with agarose gel electrophoresis and density gradient ultracentrifugation revealed that large triglyceride-rich particles were virtually lacking in interstitial fluid and that the relation between the low density lipoproteins (LDL) and high density lipoproteins (HDL) was shifted towards a greater proportion of HDL. The lipoprotein distribution in the HDL range of interstitial fluid differed from that of serum showing one maximum at a density of about 1.070 g/ml (serum HDL_2 about 1.090 g/ml) and one at a density of 1.130–1.140 g/ml (serum HDL_3, 1.110–1.120 g/ml). The former subfraction contained most of the lipoprotein-bound apoE while the latter contained the major part of apoA-I and apoA-II. Studies of the lipoproteins of interstitial fluid may add to our understanding of the development of atherosclerosis and xanthomatosis and may also provide valuable information on the permeability of the capillary membrane in normo- and pathophysiological states. — **Vessby, B., S. Gustafson, M. J. Chapman, K. Hellsing, and H. Lithell.** Lipoprotein composition of human suction-blister interstitial fluid. *J. Lipid Res.* 1987. **28:** 629–641.

Supplementary key words lipids • high density lipoproteins • apolipoproteins • capillary permeability

Considerable uptake and degradation of low density lipoproteins takes place in extrahepatic tissues, for example in skin fibroblasts and peripheral smooth muscle cells (1). There is an uptake into the cells from and exchange of lipoproteins with the interstitial fluid surrounding these cells. During the passage from the circulation to the extravascular space the lipoprotein particles have to pass the endothelium and the capillary basement membrane. Cholesterol deposited or synthesized in extrahepatic tissues must be removed from these cells and transported to the liver to be degraded and excreted (2). In the process of the reverse cholesterol transport, the interstitial fluid lipoproteins should play an important role. It has been suggested that the high density lipoproteins (HDL) in the interstitial fluid are the preferential acceptors for cholesterol from extravascular cells in vivo (3, 4).

There are few reports in the literature where human interstitial fluid lipoproteins have been characterized. Reichl and coworkers (3–8) have in several studies investigated the lipoproteins of human peripheral lymph as a model of interstitial fluid lipoproteins. We have utilized a technique (9), used earlier mainly in dermatological research, to obtain interstitial fluid by applying mild suction to the skin. The suction-blister fluid thus obtained is considered to be representative of interstitial fluid of the total body (10, 11). The present report concerns the lipid and apolipoprotein concentrations and distributions of the interstitial fluid from apparently healthy men and women compared with the corresponding serum lipoprotein lipid and apolipoprotein concentrations.

It has been reported that peripheral lymph contains a higher proportion of large relative to small HDL particles than plasma (4) with several populations of apolipoprotein A-I containing lipoproteins larger than the plasma HDL_2. Also, the composition of apolipoprotein B-containing lipoproteins in peripheral lymph has been reported to differ from that of plasma (6). In the present

Abbreviations: IF, interstitial fluid; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

[1]This work was presented in part at the 7th International Symposium on Atherosclerosis, Melbourne, Australia, October 1985.

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study we made an attempt to further characterize the lipoprotein composition and distribution in interstitial fluid, especially that of HDL, by density gradient ultracentrifugation.

MATERIALS AND METHODS

The interstitial lipid and apolipoprotein concentrations were determined in 12 men, mean age 38.2 years (range 25–51) and 15 women, mean age 38.6 years (range 21–60). They were apparently healthy and took no medication. They were all on their habitual diets.

Interstitial fluid was obtained as described by Kiistala (9) by applying mild suction (200–250 mm Hg) on the skin using transparent plastic cups containing an adapter plate with five small holes (Dermovac®, Instrumentarium, Helsinki, Finland) and collecting the fluid (150–300 μl) in the blisters thus formed after approximately 2 hr. The cups were applied either on the midvolar forearm or on the paraumbilical region of the abdomen. The blister fluid was collected by careful suction into syringes equipped with Mantoux needles. The fluid was directly transferred into small sealed vials and rapidly put into the freezer. The samples were kept at −70°C until analyses of lipid and apolipoprotein concentrations were made. Agarose gel electrophoresis was performed on fresh samples kept at 4°C until analysis.

For density gradient ultracentrifugation, larger volumes of blister fluid (0.45–0.8 ml) were collected in three subjects (for clinical characteristics see below). In these subjects several suction cups were applied on the paraumbilical region of the abdomen and all blister fluid from each person was pooled before centrifugation. Gentamicin at a concentration of approximately 100 μg/ml was added both to the blister fluid and to the corresponding serum samples to avoid bacterial growth during the centrifugation procedures.

Blood was drawn after a 12–14-hr overnight fast. Venous blood samples were allowed to clot at room temperature and serum was isolated by low speed centrifugation. EDTA was added as a 5% solution to serum at a final concentration of 0.05%. Triglyceride and cholesterol concentrations were determined in serum, interstitial fluid, and in the isolated lipoprotein fractions by enzymatic methods using Boehringer-Mannheim (Munich, FRG) kits 126012 and 124087, modified for use in a Multistat III F/LS apparatus (Instrumentation Laboratories, Lexington, MA). Very low density lipoproteins (VLDL) were isolated as the top fraction after ultracentrifugation of serum at d 1.006 g/ml for 16 hr at 15°C at 105,000 g in a Beckman L2-65B preparative ultracentrifuge using a 40.3 rotor as described by Havel, Eder, and Bragdon (12). The infranate (d > 1.006 g/ml) was treated with sodium phosphotungstate and magnesium chloride to precipitate low density lipoproteins (LDL) leaving HDL in the supernatant (13). The concentrations of the lipids in LDL were obtained indirectly by subtracting the HDL lipid levels from the lipid concentrations of the bottom fraction after centrifugation at d 1.006 g/ml. The top and bottom fractions after centrifugation at d 1.006 g/ml, as well as serum, were analyzed by agarose gel electrophoresis (14). Densitometric scans of Sudan Black-stained agarose gels were performed on a Quick Scan Jr. apparatus (Helena Laboratories, Beaumont, TX).

Apolipoproteins (apo) B, A-I, and A-II were quantified by electroimmunoassay using monospecific antibodies (15). The blister fluid samples produced rockets with a shape similar to that of serum, and the interstitial fluid dilution curves showed the same slopes as the standard curves. Reference standards for calibration of the electroimmunoassays for apoB, A-I, and A-II were generously provided by Dr. P. Alaupovic, Oklahoma Medical Research Foundation, Oklahoma City, OK. ApoC-II, C-III, and E were determined by radioimmunoassay (16, 17) as previously described. Different dilutions of blister fluid produced displacement curves with the same slopes as the standard curves.

Serum samples and blister fluid from three subjects were also submitted to density gradient ultracentrifugation using a discontinuous step gradient based on NaCl/KBr solutions varying in density from 1.06 to 1.24 g/ml. The separation was achieved after a single ultracentrifugation at 15°C for 48 hr at 40,000 rpm (56.7×10^7 g_{av}-min) in a swinging bucket rotor (18).

Albumin, α-1-antitrypsin, orosomucoid, haptoglobin, immunoglobulin G, A, and M were analyzed in blister fluid and serum using an immunoturbidimetric technique in a centrifugal analyzer (Multistat, Instrumentation Laboratories). Antisera were purchased from Atlantic Antibodies (Scarborough, ME) and Dakopatts (Denmark). The methods were standardized using Seronorm Protein (Nycomed, Norway). The corresponding blister fluid and serum samples were analyzed in the same analytical run.

Informed consent was obtained from all subjects from whom interstitial fluid and blood were collected.

Statistics

Means, standard deviations, and Pearson product-moment correlation coefficients were calculated by ordinary methods. Relationships between dependent variables and explanatory variables were also estimated by the stepwise regression technique. We are indebted to Dr. I. Selinus for performing the statistical calculations.

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RESULTS

Lipid and apolipoprotein concentrations in serum and in interstitial fluid

The serum lipoprotein lipid concentrations are shown in **Table 1.** Of the 27 apparently healthy subjects, 3 of the males and 1 of the females showed lipoprotein lipid levels above our cut-off points for normality (VLDL triglycerides, 1.4 and 1.0 mmol/l, and LDL cholesterol 5.2 and 5.7 mmol/l for men and women, respectively, corresponding to the 85th percentile in a local control material) (19). One subject had a VLDL triglyceride concentration of 6.33 mmol/l explaining the large variation of this lipid. The other three subjects had only slightly elevated lipid levels.

The lipid and apolipoprotein concentrations in serum and in interstitial fluid are given in **Table 2.** The lipid and apolipoprotein concentrations were generally somewhat higher in the abdominal samples than in those from the forearm. There was a highly significant correlation between the individual cholesterol ($P = 0.001$), apoC-II ($P < 0.001$), apoC-III ($P = 0.001$), apoE ($P < 0.001$), apoA-I ($P < 0.001$), apoA-II ($P < 0.01$), and apoB ($P < 0.01$) concentrations on the arm and the abdomen, respectively. In a small subgroup of the subjects (n = 4), free cholesterol, esterified cholesterol, and phospholipid concentrations in serum and in abdominal blister fluid were also measured. In all samples the relative proportion of esterified cholesterol was higher in serum than in the interstitial fluid, with a ratio between esterified and free cholesterol that was 22% lower in the blister fluid. Similarly, the phospholipid/free cholesterol ratio was 19% lower.

Relationships between interstitial fluid and serum lipid and apolipoprotein concentrations

The ratios between forearm interstitial fluid and serum (IF/S ratio) for cholesterol and for apolipoprotein concentrations were generally lower than those between the abdominal fluid and serum (**Table 3**). However, for each location, the IF/S ratio was always lowest for apoB, apoC-II, and apoE and highest for apoA-I and apoA-II. Consequently the ratio apoA-I/apoB was lower in serum than in the interstitial fluid ($P < 0.001$, compared with blister fluid from the arm as well as with abdominal fluid). The apoC-II/apoC-III ratio was higher in the interstitial fluid than in serum; the apoC-II/apoC-III ratio in the abdominal fluid was significantly higher when compared with that of serum ($P < 0.01$), while the arm-serum difference was not quite statistically significant ($P = 0.06$). The apoC-III/apoE ratio was lower in the interstitial fluid than in serum (significant arm-serum, $P < 0.01$), while the apoA-I/apoA-II ratio was similar.

The relationships between interstitial fluid lipid and apolipoprotein concentrations and serum lipoprotein and apolipoprotein concentrations, as estimated by a stepwise regression technique, are shown in **Table 4.** The cholesterol concentration in interstitial fluid was most significantly correlated to the serum HDL cholesterol concentration, but was also significantly correlated to the LDL cholesterol concentration. For all the interstitial fluid apolipoproteins, the main correlations were to the serum HDL cholesterol concentrations while no sig-

TABLE 2. Lipid (mmol/l) and apolipoprotein (mg/dl) concentrations in serum and in interstitial fluid in apparently healthy men and women

Fraction	Sex[a]	Serum	Interstitial Fluid	
			Arm	Abdomen
TG[b]	M	1.97 ± 1.79	0.24 ± 0.06	0.35 ± 0.05
TG	F	1.24 ± 0.43	0.32 ± 0.03	0.32 ± 0.09
Chol	M	5.23 ± 1.08	0.87 ± 0.25	1.19 ± 0.41
Chol	F	5.40 ± 1.10	1.13 ± 0.25	1.30 ± 0.27
ApoA-I	M	128 ± 15	26 ± 10	35 ± 10
ApoA-I	F	138 ± 15	32 ± 6	39 ± 8
ApoA-II	M	74 ± 13	16 ± 5	22 ± 6
ApoA-II	F	75 ± 11	18 ± 4	22 ± 5
ApoB	M	108 ± 31	15 ± 6	21 ± 7
ApoB	F	118 ± 31	19 ± 5	22 ± 5
ApoC-II	M	5.8 ± 2.3	0.77 ± 0.31	0.98 ± 0.38
ApoC-II	F	4.7 ± 1.3	0.76 ± 0.25	0.91 ± 0.20
ApoC-III	M	14.4 ± 5.9	2.06 ± 0.97	2.96 ± 1.18
ApoC-III	F	12.1 ± 2.5	2.32 ± 0.56	2.95 ± 0.81
ApoE	M	4.6 ± 2.9	0.44 ± 0.30	0.68 ± 0.34
ApoE	F	3.8 ± 1.8	0.57 ± 0.39	0.81 ± 0.48

[a] Men, n = 12; women, n = 15.
[b] TG analyses of interstitial fluid were performed in only eight subjects, five men and three women.

TABLE 1. Serum lipoprotein lipid concentrations in apparently healthy men and women

Subjects (n)	VLDL		LDL		HDL	
	TG	Chol	TG	Chol	TG	Chol
	mmol/l ± SD					
Men (12)	1.35 ± 1.64	0.68 ± 0.85	0.36 ± 0.13	3.42 ± 1.01	0.23 ± 0.09	1.13 ± 0.26
Women (15)	0.56 ± 0.24	0.31 ± 0.16	0.39 ± 0.14	3.78 ± 0.98	0.26 ± 0.07	1.34 ± 0.18

VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; TG, triglycerides; Chol, cholesterol concentration.

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TABLE 3. Ratios (IF/S) between cholesterol and apolipoprotein (apo) concentrations in interstitial fluid from arm and abdomen, respectively, and serum

	Arm/Serum	Abdomen/Serum
	mean ratio ± SD	
Cholesterol	0.19 ± 0.05	0.24 ± 0.06
ApoC-II	0.16 ± 0.07	0.20 ± 0.06
ApoC-III	0.18 ± 0.07	0.24 ± 0.08
ApoE	0.14 ± 0.10	0.22 ± 0.14
ApoA-I	0.22 ± 0.05	0.28 ± 0.05
ApoA-II	0.23 ± 0.05	0.29 ± 0.06
ApoB	0.16 ± 0.07	0.20 ± 0.07

nificant relationships at all were seen to VLDL lipid levels. No significant correlations were seen between the apoE concentrations in interstitial fluid and the serum lipoprotein lipid or apolipoprotein concentrations. For all variables the relationships between the dependent variable and the explanatory variables were stronger in the abdominal than in the arm blister fluid.

Figs. 1A and B show the relationships between the concentrations of apoA-I in abdominal skin interstitial fluid and apoA-I and HDL cholesterol in serum, respectively. It is apparent that the correlation between apoA-I in interstitial fluid and HDL cholesterol in serum is stronger than that between the apoA-I concentration in interstitial fluid and serum. Similar relationships were seen for apoA-I in blister fluid from the arm and apoA-I and HDL cholesterol in serum, respectively. Also for apoA-II in interstitial fluid, the relationship to HDL cholesterol is more direct than to apoA-II in serum (**Figs. 2A and B**).

There was virtually no correlation between interstitial fluid apoB and serum apoB (**Fig. 3A**) and a very weak correlation between the apoB in interstitial fluid and the LDL-cholesterol in serum (Fig. 3B). The same picture was seen with regard to the correlations between arm interstitial fluid and serum. Fig. 3B clearly shows that a certain apoB concentration in the interstitial fluid may correspond to a wide range of serum LDL-cholesterol concentrations.

The ratio between apoA-I and apoB in interstitial fluid was on the average considerably higher than in serum but there was also a scatter of the observations indicating that a certain apoA-I/apoB ratio in serum might correspond to different apoA-I/apoB ratios in the interstitial fluid (**Fig. 4A**). This was especially true for low apoA-I/apoB ratios in serum. Fig. 4B shows the relationship between the apoA-I/apoB ratios in interstitial fluid from arm and abdomen, respectively, indicating a high degree of correlation between the ratios measured on these two locations. The r value is somewhat lower than in Fig. 4A due to the outlier. Otherwise the resemblance between the ratios is striking. The similarity of these ratios measured in interstitial fluid on two different locations in the same individual indicates that it may be possible to demonstrate reproducible inter-individual differences in the gradient between serum and interstitial fluid.

Agarose gel electrophoresis

The lipoprotein distribution after agarose gel electrophoresis, as shown in **Fig. 5,** showed distinct differences in the blister fluid and serum. The slow moving band corresponding to the LDL (beta band) was much fainter than in serum and the VLDL (pre-beta band) was virtually

TABLE 4. Relationships between interstitial fluid lipid and apolipoprotein concentrations, respectively, and serum lipoprotein and apolipoprotein concentrations as estimated by a stepwise regression technique

Dependent Variable	Interstitial Fluid	Intercept	Explanatory Variables (Serum)	r^2
TG	Arm		ns (not significant)	
TG	Abdomen	0.17	HDL TG (0.77*)	0.53
Chol	Arm	−0.17	HDL Chol (0.13**), LDL Chol (0.56**)	0.48
Chol	Abdomen	−0.46	HDL Chol (0.12***), LDL Chol (1.01**)	0.67
ApoC-II	Arm		ns	
ApoC-II	Abdomen	−0.27	HDL Chol (0.76***), C-II (0.27***), TG (−0.29***), B (−0.005**)	0.59
ApoC-III	Arm	0.42	HDL Chol (1.44*)	0.20
ApoC-III	Abdomen	−0.75	HDL Chol (2.97***)	0.53
ApoE	Arm		ns	
ApoE	Abdomen		ns	
ApoA-I	Arm	3.45	HDL Chol (20.86***)	0.36
ApoA-I	Abdomen	−8.85	HDL Chol (24.42***), B (−0.18*), A-II (0.18*)	0.79
ApoA-II	Arm	4.62	HDL Chol (9.86**)	0.25
ApoA-II	Abdomen	−0.32	HDL Chol (8.65*), LDL TG (−22.26**), A-II (0.13*), HDL TG (24.18*)	0.71
ApoB	Arm	6.91	LDL Chol (2.83**)	0.24
ApoB	Abdomen	−5.79	HDL Chol (16.20***), LDL Chol (1.99*)	0.56

The limits for exclusion and inclusion of variables were set to $P < 0.15$. Estimates of regression coefficients are reported for the explanatory variables included with P values < 0.05 (*, **, and ***, $P < 0.05$, 0.01, and 0.001, respectively). The explanatory variables are listed in their order of inclusion in the equations. The proportion of the variation of the dependent variable explained by the selected model is indicated by r^2. Abbreviations are as in Tables 1 and 2.

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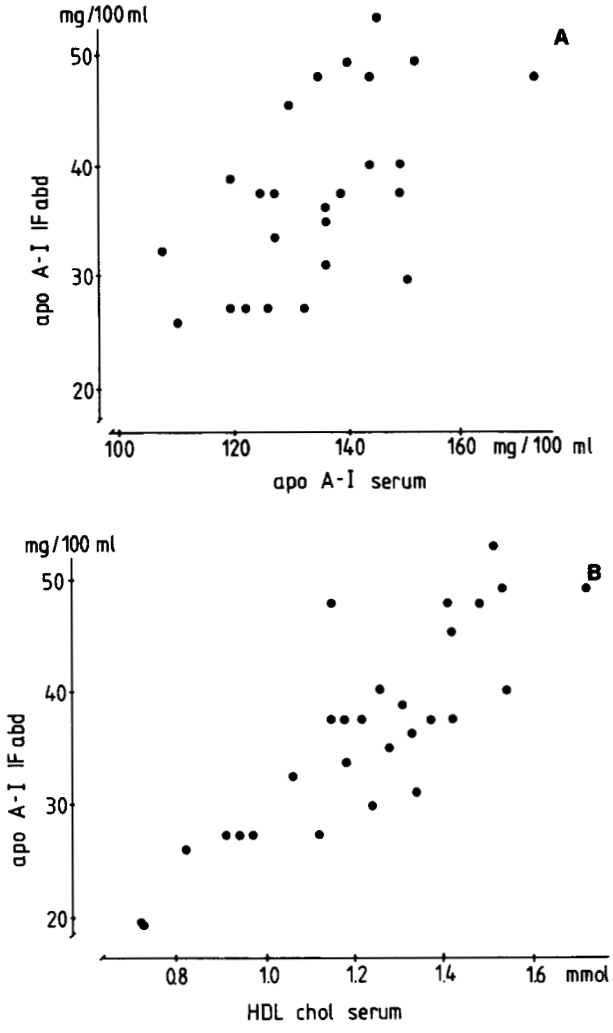

Fig. 1. Relationships between the concentrations of apoA-I in abdominal interstitial fluid (IF abd) and in serum (A: $r = 0.65$, $P < 0.001$) and serum HDL cholesterol (B: $r = 0.83$, $P < 0.001$), respectively.

absent in the blister fluid, even when pronounced in serum.

Regarding HDL, there were no apparent differences in mobility but there was a clear tendency to diverging distributions of the two HDL fractions in the blister fluid and in the serum. This was verified by densitometric scanning (**Fig. 6**) of the agarose gels. These gels showed not only an altered distribution of the different lipoprotein fractions in blister fluid compared with serum, but also a different shape of the HDL lipoprotein fraction in interstitial fluid. The main difference compared with serum seemed to be a proportionally higher peak of the faster moving, more charged particles in the blister fluid.

Density gradient ultracentrifugation

In three subjects a density gradient ultracentrifugal procedure was used to determine the lipid and apolipoprotein distributions in different lipoprotein fractions according to hydrated density. The clinical characteristics and the serum lipoprotein lipid and apolipoprotein concentrations of these subjects are given in **Table 5.**

The lipoprotein lipid and apolipoprotein profiles in serum and blister fluid are shown in **Fig. 7A–C.** While the density profiles used for separation of serum and blister fluid were identical, the volumes of the fractions collected were different. The serum was fractionated according to the detection and positioning of the various lipoprotein bands but no such bands were visible in the interstitial fluid. Consequently, these samples were subfractionated into consecutive 0.5-ml portions. This gives a somewhat better resolution of the profiles, especially in the HDL region, for the interstitial fluid lipoproteins than for the serum lipoproteins as shown in the figures. Empirically,

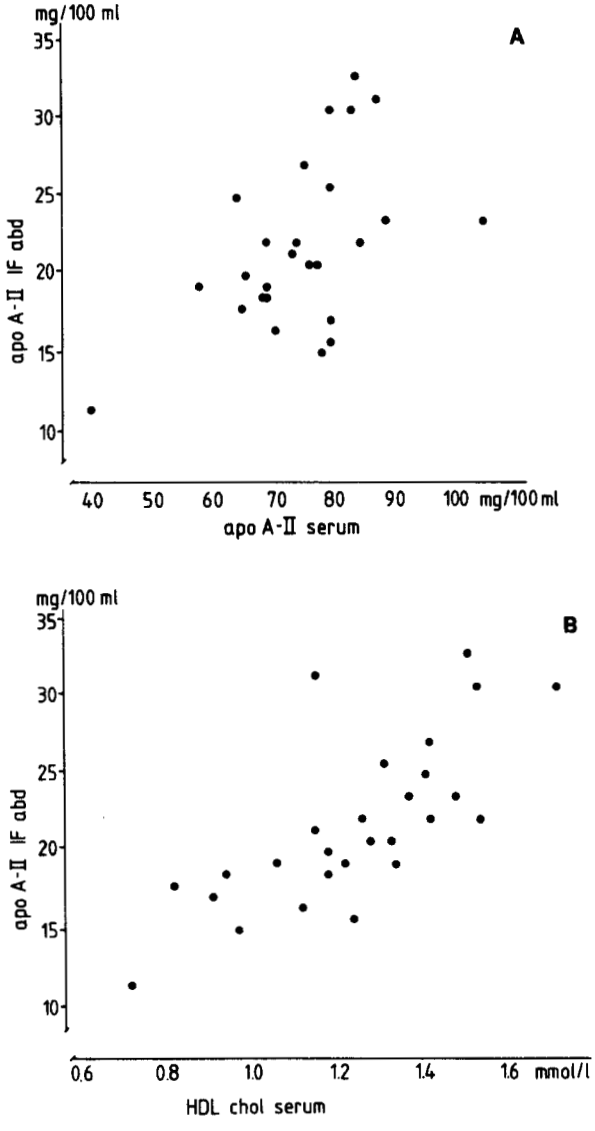

Fig. 2. Relationships between apoA-II in abdominal interstitial fluid (IF abd) and serum apoA-II (A: $r = 0.54$, $P < 0.01$) and serum HDL cholesterol (B: $r = 0.73$, $P < 0.001$), respectively.

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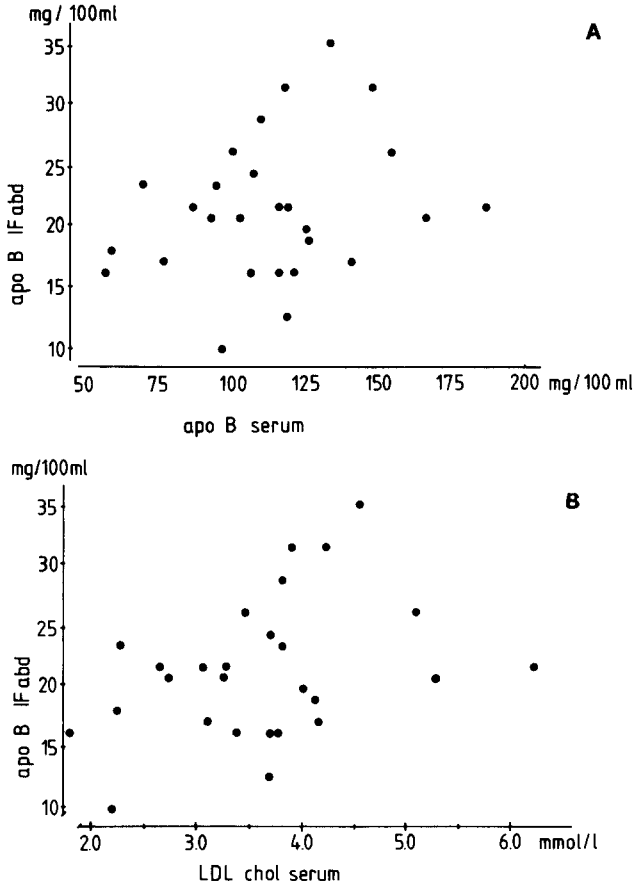

Fig. 3. Relationships between apoB in abdominal interstitial fluid (IF abd) and serum apoB (A: r = 0.27, not significant) and serum LDL cholesterol (B: r = 0.37, not significant), respectively.

with this technique, the peak of the serum HDL_2 and HDL_3 fractions, respectively, correspond to densities of approximately 1.09 and 1.12 g/ml (18).

The mean recoveries during the density gradient procedure were 95% for serum cholesterol, 86% for serum apoB, 73% for apoA-I, and 59% for apoA-II. The IF/S ratios for cholesterol in subjects 1 and 2 (0.22 and 0.19), apoB (0.20 and 0.15), apoA-I (0.24 and 0.21), and apoA-II (0.29, 0.24) were similar to those found earlier when the total concentrations of lipids and apolipoproteins in interstitial fluid and serum were measured (Table 3). This indicates that the recoveries of interstitial fluid and serum during the density gradient centrifugation were of similar magnitude in spite of the considerably lower lipid and apolipoprotein concentrations in interstitial fluid.

Although the recoveries of apoC-III and apoE could not be exactly calculated the estimated figures seemed reasonable. However, in contrast to the lipid and the apoB, A-I, and A-II profiles, the distribution of apoE and C-III consistently showed a part of these apolipoproteins to separate in the density region greater than 1.21 g/ml. This suggested that apolipoproteins may have been stripped off from the lipoprotein surfaces during the density gradient procedure. Thus, 30–40% of the apoC-III and E in serum were found in this density region in patients 1 and 2 while approximately 10% of the apoC-III was found in this region in patient 3. In the interstitial fluid 40–50% of the apoC-III and E were found in density fractions > 1.20 g/ml in all three patients.

The IF/S ratios for cholesterol and apolipoproteins in patient 3 were considerably lower than those in patients 1 and 2, as could be expected, as much of the lipid and apolipoprotein material in serum was transported in large lipoprotein particles which should penetrate the capillary membrane very poorly.

Looking at the density gradient profiles of the three subjects (Fig. 7) some differences between serum and interstitial fluid are clearly visible. Although the resolution of the serum profile was not as good as that of interstitial fluid it was still quite obvious that the major lipoprotein peak in the HDL density region in the interstitial fluid in all patients had a density that was clearly higher than that of serum. While the major HDL peak in serum, apparently

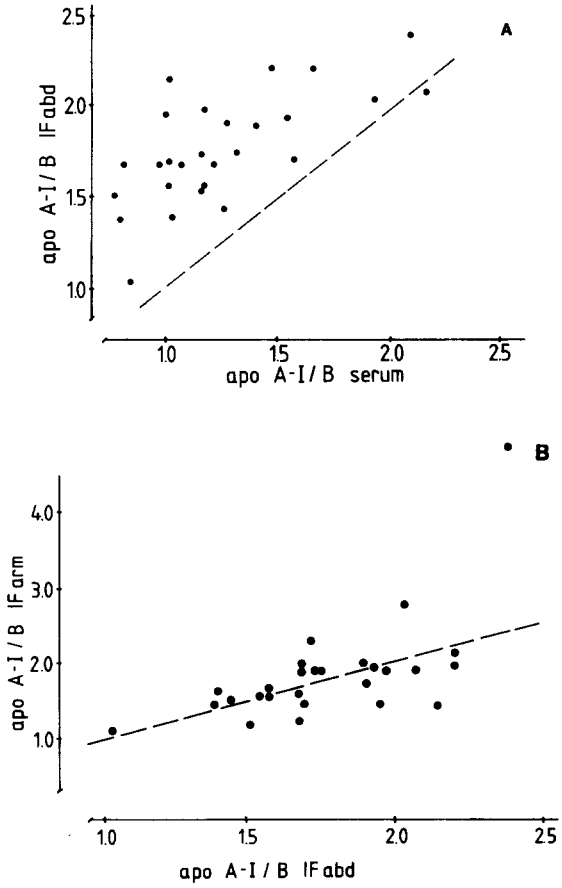

Fig. 4. A. Relationship between the apoA-I/apoB ratio in abdominal interstitial fluid (IF abd) and serum (A: r = 0.69, $P < 0.001$). B. Relationship between the apoA-I/apoB ratio in interstitial fluid from the forearm (IF arm) and from the abdomen (B: r = 0.61, $P < 0.001$). The broken line indicates X = Y.

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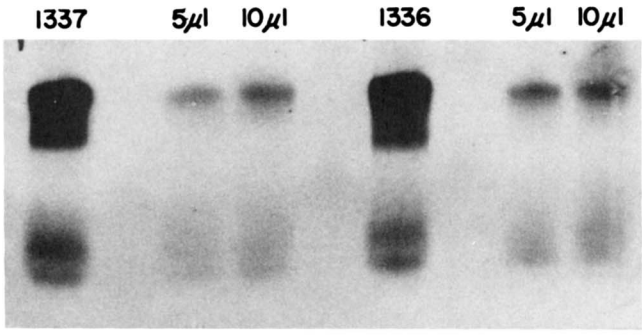

Fig. 5. Agarose gel electrophoresis of two serum samples (1337 and 1336) and corresponding interstitial fluid (5 and 10 μl applied to the gel), respectively.

corresponding to HDL_3, determined from the cholesterol, apoA-I, or apoA-II profile had a density of 1.11–1.12 g/ml, the major HDL peak of the interstitial fluid lipoproteins had a higher density of about 1.13–1.14 g/ml. Actually, in the interstitial fluid there was, in all patients, a clear tendency to a bimodal distribution where the light part of the HDL distribution had a maximum at lower densities than the serum HDL_2 peak (with this technique corresponding to a density of approximately 1.09 g/ml) of about 1.060–1.080 g/ml). This subfraction, with a maximum of lower density than that of ordinary HDL_2, was clearly apparent in at least two of the subjects (subjects 2 and 3). These different distributions seem to be compatible with the differences indicated by the scans of the agarose gel electrophoresis (Fig. 2).

The profiles of apoC-III and E have to be regarded with care as much of the material evidently was stripped off during the centrifugation procedure. However, a part of the material recovered in the fraction with $d > 1.21$ g/ml probably originates from the larger, triglyceride-rich lipoproteins with densities less than 1.020 g/ml. With regard to the distribution of apoE and apoC-III within the HDL density range, the major apoE peak in interstitial fluid was, in all patients, around a density of 1.070 g/ml with a tendency to a wider distribution in serum. ApoC-III seems to have a maximum with a somewhat higher hydrated density than apoE both in serum and interstitial fluid.

DISCUSSION

The present study was undertaken as an effort to obtain new information on the lipoprotein composition of human intersitial fluid. Earlier, Reichl et al. (3–8) undertook a series of studies to characterize lipoprotein constituents of human peripheral lymph, which is supposed to mirror the interstitial fluid composition (20). Other workers have studied the composition of thoracic duct lymph (21, 22), ascitic fluid (23), and pleural effusions (24). The composition of peripheral lymph may show regional differences and is also influenced by the rate of flow in the vessel studied (25). The composition of thoracic duct lymph is, of course, influenced by the dietary fat intake to a major degree.

Direct studies of interstitial fluid lipoprotein composition may presumably give us additional information on cellular lipoprotein metabolism, possibly reflecting a somewhat earlier stage in the sequence of metabolic events than the peripheral lymph. The lipoprotein particles are transported through the capillary wall via the interstitial fluid bathing the tissue cells and into the lymphatic channels. Some of the lipoproteins that reach the interstitial fluid may return directly to the blood stream through the walls of the capillaries. However, a larger proportion returns to the circulation via the lymphatic system. Based on studies of the distribution of cholesterol and apolipoprotein A-I between the lipoproteins in plasma and peripheral lymph, it has been suggested that small HDL particles, after passage of the capillary walls, are converted to larger HDL in interstitial fluid by incorporation of cholesterol and other lipids from extravascular cells (3, 4).

Subepidermal blisters can be obtained when low pressure suction is maintained for sufficiently extended periods (9). The fluid within the blisters is water-clear and acellular. It contains neither red cells nor inflammatory cells. There is no resulting scarring.

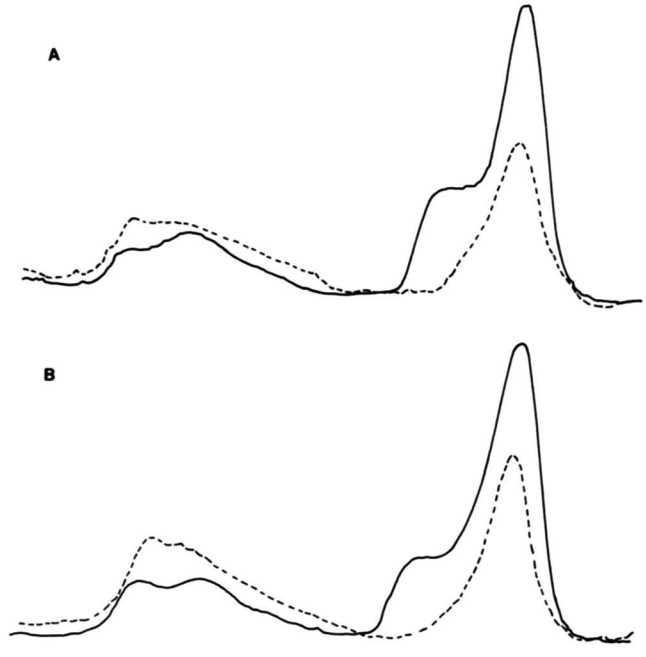

Fig. 6. Densitometric scans of the Sudan Black-stained agarose gel shown in Fig. 5. The scans of the serum samples (Figs. 6A = 1337, Fig. 6B = 1336) are shown by solid lines, and those of the corresponding interstitial fluid by broken lines. The scans of the interstitial fluid are amplified to be comparable with those of serum.

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TABLE 5. Clinical characteristics and serum lipoprotein lipid and apolipoprotein concentrations of the subjects analyzed with density gradient centrifugation

			Lipoprotein Serum Lipid Concentrations								Serum Apolipoprotein Concentrations			
			VLDL		LDL		HDL		Serum					
Subject	Sex	Age	TG	Chol	TG	Chol	TG	Chol	TG	Chol	B	A-I	A-II	History
		yr	*mmol/l*								*mg/dl*			
1	F	31	0.75	0.36	0.41	3.40	0.16	1.04	1.36	4.63	99	129	72	Healthy
2	M	30	0.62	0.25	0.29	2.77	0.13	0.84	1.06	3.73	90	103	67	Healthy
3	M	69	2.90	1.04	0.68	5.58	0.12	0.72	3.86	7.02	200	95	60	Diabetes + coronary heart disease + type IIb hyperlipoproteinemia

Several studies have concluded that suction-blister fluid obtained from normal skin using the present technique represents interstitial fluid (10, 11). It has been suggested that suction-blister fluid can be regarded as representative of the average composition of the interstitial fluid of the total body, supported by the fact that the distribution of ratios of several molecules are similar to those previously obtained in long-term metabolic turnover studies (26). It has also been shown that suction blisters of the skin behave like a compartment with physiological dynamic interstitium-like properties (27). The suction blister technique represents a relatively noninvasive, noninflammatory way of collecting fluid from the skin interstitium with the possibility of direct measurement of the concentrations of various tracers. The concentration of high molecular weight substances in blister fluid seems to be determined mainly by passive diffusion, which is in agreement with the findings of Garlick and Renkin (28) for peripheral lymph. The capillary system remains intact under the conditions used to achieve the blister fluid without any demonstrable increase of the permeability of the capillaries in the blister bottom (10, 27). The reproducibility of the method seems to be good (29), which is also supported by the present findings of strong positive correlations between the concentrations of lipids and apolipoproteins in blister fluid obtained from different locations in the same individuals. Regardless of the site of the abdominal skin investigated, Staberg et al. (29) did not find any variation in the appearance of labeled albumin or the concentration of different proteins.

Reichl and coworkers (5) have reported the presence of apoA, apoB, and apoC in human peripheral lymph, but with a distribution in density fractions different from that in plasma. Also, it was reported that iodine-labeled apolipoproteins, after intravenous injection, could be recovered in lymph lipoproteins similar to plasma high density and low density lipoproteins (6). The results suggested that apolipoproteins of the plasma lipoproteins reached the interstitial fluid and that some lipoproteins undergo modification during the passage into peripheral lymph. Vermeer, Reman, and van Gent (10) could demonstrate the presence of apoA-I and B in suction-blister fluid obtained with the present technique with ratios between blister fluid and serum very similar to those reported in the present work. Corresponding ratios reported by Reichl and Myant (7) for peripheral lymph are somewhat lower than those reported by Vermeer et al. (10) and by us in the suction-blister fluid. We have now extended the studies to a larger group of apparently healthy males and females, where we have also determined the concentrations of apoA-II, C-II, C-III, and E (Table 2).

Determination of different proteins with varying size and molecular weight in suction-blister fluid and serum has demonstrated an inverse relationship between molecular weight and blister to serum concentrations of the proteins studied indicating an intact sieve function of the vascular wall (10). Compatible with this, the IF/S ratio was smaller for apoB (representative for the concentration of low density lipoproteins) than for apoA-I (representative for high density lipoproteins).

When the blister fluid/serum concentrations of different proteins were measured and plotted against the logarithm of their estimated molecular weight (**Fig. 8**), lower IF/S ratios were found with increasing molecular weight. The apparently decreased permeability of IgA and haptoglobin in relation to their estimated mean molecular weight is an interesting finding which may be related to the variation in molecular sizes characteristic for these molecules. The lower mean permeability of α-1-antitrypsin and orosomucoid than albumin may be related to conformational differences as a result of a high content of carbohydrates in the former molecules. The findings in this study agree well with those of Vermeer et al. (10), who in addition reported that insulin (molecular weight 6,600) had a blister serum concentration ratio of 0.90 demonstrating a high degree of capillary permeability for small molecules, and with the data by Garlick and Renkin (28) from a study of lymph/serum ratios for dextran.

Agarose gel electrophoresis showed virtually no lipid-stainable material in the pre-beta region (Fig. 5) indicating the near absence of VLDL lipoproteins in interstitial

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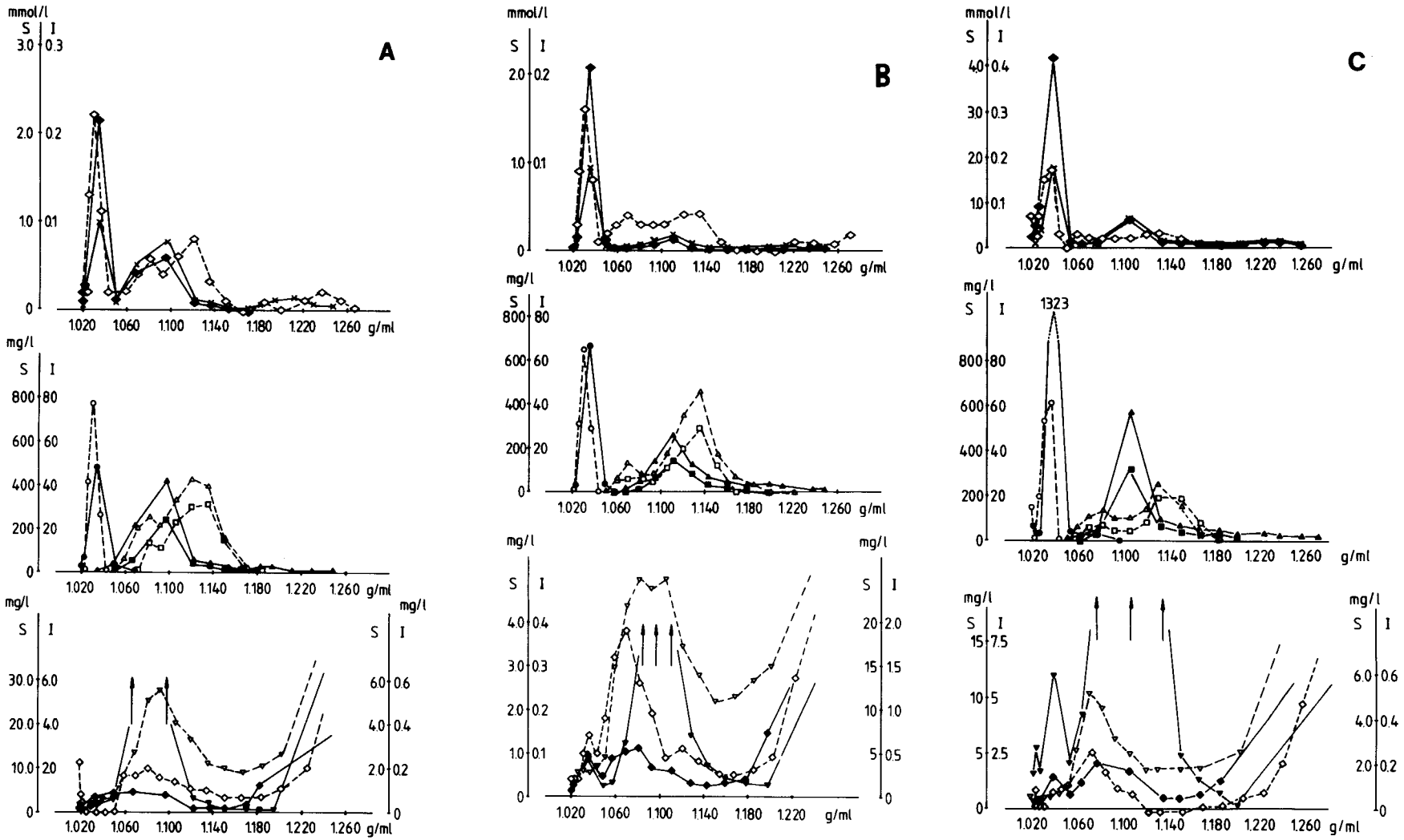

Fig. 7. Density gradient ultracentrifugation profiles of serum samples 1 (A), 2 (B), and 3 (C) and corresponding interstitial fluid samples. Upper panels: S-cholesterol (—◆—◆—); IF-cholesterol (--◇--◇--); S-phospholipids (—X—X—). Middle panels: S-apoB (—●—●—); IF-apoB (--O--O--); S-apoA-I (—▲—▲—); IF-apoA-I (--△--△--); S-apoA-II (—■—■—); IF-apoA-II (--□--□--); Bottom panels: S-apoC-III (—▼—▼—); IF-apoC-III (--▽--▽--); S-apoE (—◆—◆—); IF-apoE (--◇--◇--). Note the different scales for serum (S) and interstitial fluid (I). In the bottom panels the concentrations of apoC-III are indicated on the left side and those of apoE on the right side of the density gradient profile; ↑ denotes high values.

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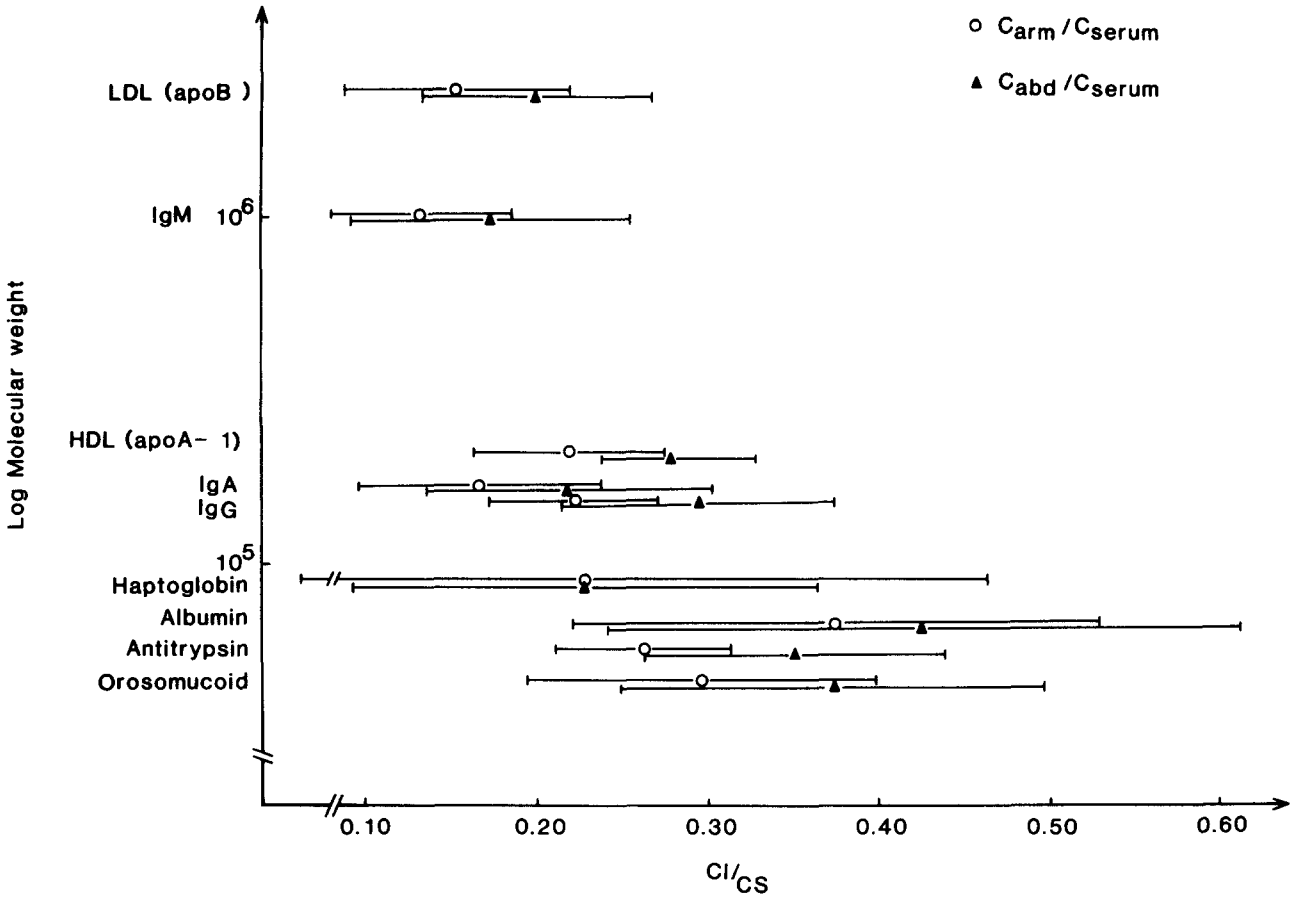

Fig. 8. Blister interstitial fluid/serum concentrations (CI/CS, mean ± SD) of different proteins plotted as a function of the logarithm of their molecular weight.

fluid. The absence of large triglyceride-rich lipoproteins in the interstitial fluid was also supported by the lack of significant correlations beteween interstitial fluid apolipoprotein and cholesterol concentrations on the one hand, and the VLDL lipid levels in serum on the other (Table 4). In a recent study of human lymphedema fluid lipoproteins Reichl et al. (30) found considerably less VLDL and intermediate density lipoproteins than in plasma.

The lack of any significant correlations between the apoE concentrations in interstitial fluid and serum (Table 4) is noteworthy. Although the major part of apoE in serum is transported in large lipoprotein particles with a poor ability to penetrate the capillary wall, another explanation for the missing correlations could be that a significant part of the interstitial fluid apoE is synthesized de novo in the extravascular tissue, e.g., by monocyte-derived macrophages (31).

The ratios between interstitial fluid and serum were generally lower on the forearm than on the abdomen (Table 3). Also, on the latter location, the correlations to the serum concentrations were stronger (Table 4). The reason for these differences, whether due to diverging capillary permeability or degree of capillarization or to differences in the extravascular tissues, is not readily apparent. However, the individual concentrations of cholesterol and apolipoproteins in the arm and abdominal blister fluid were significantly correlated and there was also a good reproducibility of the ratio between apoA-I and apoB in interstitial fluid on the two locations (Fig. 4B).

The data indicate not only that smaller molecules pass more easily to the interstitial space from the circulation than larger ones, but also that there are considerable inter-individual differences in the capillary permeability. Thus, a certain apoB concentration or LDL concentration in serum (Fig. 3) may correspond to widely different apoB concentrations in the interstitial fluid. The IF/S ratio for apoB may be related not only to the capillary permeability but also to individual differences in the size distribution of the LDL particles. Thus it is known that hypertriglyceridemia is associated with a preponderance of smaller LDL particles (32) that may penetrate more easily to the extravascular space than LDL particles of normal size.

There are also individual differences, although less pronounced, between the concentrations of apoA-I and A-II in interstitial fluid and those of the corresponding apo-

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lipoproteins and HDL in serum, respectively (Figs. 3 and 2). If there is a reproducible inter-individual difference between the permeability for apoB-containing lipoproteins, resulting in different A-I/B ratios in the extravascular space, this may be of considerable importance with regard to lipoprotein uptake in the cells and risk of developing atherosclerotic lesions.

Analyses of the lipoprotein composition and distribution in interstitial fluid and serum using agarose gel electrophoresis (Figs. 5 and 6) and density gradient ultracentrifugation (Fig. 7) clearly show differences of the lipoprotein distribution in the two compartments. The relation between LDL and HDL is shifted towards a greater proportion of HDL in interstitial fluid. This is in accordance with what could be expected based on the diffusion properties of particles of different size. However, there were not only quantitative but also clear qualitative differences between the lipoproteins in interstitial fluid and in serum. The interstitial fluid lipoproteins within the HDL density range showed two maxima which differed from serum HDL_2 and HDL_3. Thus, there was one maximum at the approximate density of 1.070 g/ml and one at 1.13–1.14 g/ml. There were rather pronounced individual differences with regard to the interstitial fluid lipoproteins with varying relative amounts of the material with lower and higher density.

It was obvious that the HDL fraction with lower density contained most (Fig 7) of the apoE connected with lipoprotein particles in interstitial fluid, while the high density fraction with density maximum of about 1.13–1.14 g/ml contained the major part of apoA-I and A-II. The seemingly high apoE/apoA ratio in the lighter HDL fraction is compatible with the possibility that these larger, less dense particles enriched in apoE may be similar to the type of particles suggested to take a part in the reverse cholesterol transport (30). This fraction can also be compared with the large HDL particle with a high cholesterol/apoA-I ratio, which was reported to carry a higher proportion of cholesterol in lymph than in plasma (2, 16). The authors suggested that the higher ratio of large to small HDL particles in lymph than in plasma may be due to conversion of small to larger HDL in interstitial fluid by incorporation of cholesterol and other lipids from extravascular cells into the smaller particles. In lymphedema, all apoE was associated with particles larger than HDL_2 in plasma (30).

HDL particles with a size larger than plasma HDL have also been reported in the interstitial fluid of control and cholesterol-fed dogs (33) associated with alterations in the lipid and apolipoprotein composition. The apoE/apoA-I and apoA-IV/apoA-I ratios were reported to be elevated compared with plasma HDL in control dogs as well as in cholesterol-fed dogs. Thus, these particles may be engaged in the reverse cholesterol transport and possibly to some extent be synthesized in peripheral tissues, e.g., by macrophages (31).

In the present study we could also demonstrate the presence of interstitial fluid lipoproteins with higher mean density than plasma HDL_3 (Fig. 7). They could represent another type of relatively lipid-poor particles which may be either selectively filtered from plasma to the extravascular space, synthesized extravascularly, or modified by interaction with the peripheral cells with exchange or transfer of certain components. Several peripheral tissue cells have been shown to contain HDL receptors (34, 35) that may be involved in this process. It may be speculated that those small protein-rich particles may be potent cholesterol acceptors and thus play an important role in the reverse transport of cholesterol from extrahepatic tissues.

The present data also suggest that there may be certain differences between the interstitial fluid, as studied by the present technique, and peripheral lymph, as studied by Reichl and coworkers (e.g., 2, 3, 16). There are some differences both in lipoprotein density distribution and lipid and apolipoprotein composition where skin interstitial fluid in several respects may represent a situation intermediate between serum and peripheral lymph. The skin interstitial fluid contains apoE-rich particles with a mean density less than that of HDL_2 in plasma. These particles do not, however, represent the dominating HDL fraction as in lymphedema fluid (30), and the major fraction of apoA-I is in interstitial fluid found in lipoproteins with higher density than in plasma as discussed above. Possibly the interstitial fluid lipoproteins represent "earlier forms" of the lipoproteins during the passage from the capillaries to the peripheral lymph. Peripheral lymph lipoproteins may represent a later stage where these particles have taken up more lipid material and apolipoprotein E as a part of the reverse cholesterol transport.

Smooth muscle cells, fibroblasts, and several other cell types have been shown to have receptors for LDL (36) as well as for HDL (34, 35). These cells are exposed not to plasma lipoproteins but to the lipoproteins present in the interstitial fluid with a very different concentration and distribution than in serum. The present study has also clearly shown a pronounced individual variation with regard to the interstitial fluid lipoprotein concentrations which is not directly mirrored by the lipoprotein composition of serum. Individual differences in capillary permeability and transport rates may cause very diverging lipoprotein concentrations and profoundly affect the lipoprotein metabolism in extravascular tissues.

If the present technique can be used to study, in a reproducible way, the individual differences in noninflammatory interstitial fluid lipoprotein composition, this should be a valuable tool to increase our knowledge regarding the development of atherosclerosis and xanthomatosis. If there are significant individual differences in the interstitial fluid lipoprotein composition, which do not only reflect the composition of serum, it may be anticipated that, for example, studies of the ratio between A-I and B in interstitial fluid may give additional information

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about the risk of developing atherosclerotic disease. We are presently investigating whether different blister fluid/serum ratios may be seen in patients with atherosclerotic disease, hyperlipoproteinemia, or diabetes than in healthy subjects. It may be interesting to evaluate not only the possible influence of the diabetic state but also of smoking and hypertension on the permeability of the capillary membrane, studied as the interstitial fluid/serum difference of certain proteins and lipoprotein particles.

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