Lipid transfer between human serum high density lipoproteins and egg yolk lipoproteins in incubation mixtures

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ABSTRACT Ultracentrifugally isolated human serum high density lipoproteins of d 1.063–1.21 (HDL) were incubated with egg yolk lipoproteins of d < 1.006 for up to 24 hr at various concentrations. Transfer of HDL cholesterol esters to egg yolk lipoproteins occurred simultaneously with transfer of glycerides from egg yolk lipoproteins to HDL. These observations show that exchange of lipids can take place between lipoproteins in the absence of other serum proteins and enzymes. The mole ratios of HDL cholesterol esters to glycerides approached an integral value of 1:1 during the course of the incubation. These results suggest that lipid components form complexes within the HDL structure.

KEY WORDS lipoproteins · human plasma high density · egg yolk · incubation · lipid transfer · cholesterol esters · glycerides · mole ratios · complex formation

THE EFFECT of high concentrations of very low density lipoproteins (VLDL, d < 1.006) on lipid transfer between the major lipoprotein classes of incubated human serum has been described (1). This effect consisted primarily of a transfer of some cholesterol esters from the LDL (low density lipoproteins, d 1.006–1.063) and HDL (high density lipoproteins, d 1.063–1.21) to the VLDL. At the same time some glycerides were reciprocally transferred from the VLDL to the LDL and HDL. The additional cholesterol esters appearing in the VLDL apparently came from two different sources: (*a*) the cholesterol esters formed by the action of serum fatty acid transferase (2) during the incubation, and (*b*) the cholesterol esters which were initially part of the LDL and HDL lipid moieties.

As a consequence of the transfer of lipid, lipoprotein composition altered, but as there was no obvious disruption of lipoprotein structure leading to denaturation or fragmentation, we concluded that new stable lipid–lipid and possibly lipid–protein associations had been formed within the original lipoprotein structure. More quantitative information on such changes in composition may reveal what associations may have been formed. In order to avoid the complications due to the presence of other lipoproteins, proteins, and enzymes (e.g., serum fatty acid transferase) we have incubated isolated HDL with low density lipoproteins, d < 1.006, ultracentrifugally isolated from egg yolk (designated ELP). ELP were used because of their ready availability and high content of reproducibly obtainable lipoproteins with densities in the same range as VLDL from human serum. Lipid transfer between these two lipoproteins was studied as a function of lipoprotein concentration and time.

EXPERIMENTAL METHODS

Preparation of ELP

Three egg yolks were separated from the egg whites and suspended in 200 ml of saline (1.175% w/v) containing *p*-hydroxymercuribenzoate (0.338 mg/ml). All saline and buffer solutions used throughout this investigation were prepared with deionized distilled water to avoid oxidative degradation of lipoproteins promoted by the presence of cupric ions. The yolk membranes and associated materials were removed by sedimentation for 30 min at approximately 700 × *g*. The yellow supernatant solution was drawn off and subjected to preparative ultracentrifugation for 24 hr at 114,400 × *g* and 15–17°C.

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; LP, lipoproteins; ELP, egg yolk lipoproteins.

The gel of lipoprotein which collected at the top of the preparative tube was carefully removed with a spatula and dissolved in phosphate buffer (pH 7.35 and μ 0.1), containing penicillin (0.12 mg/ml) and streptomycin (0.38 mg/ml), to yield solutions of appropriate concentrations for the incubation experiments.

Preparation of HDL

Blood (250 ml) was drawn aseptically from each of three normal fasting women (ages 21–26). Serum was separated by centrifugation and then pooled. *p*-Hydroxymercuribenzoate (0.338 mg/ml), penicillin (0.12 mg/ml), and streptomycin (0.38 mg/ml) were added to the pooled serum. The HDL were ultracentrifugally isolated by procedures previously described (3). The HDL were dialyzed first against the saline solution used for suspension of the egg yolks; then against the same saline solution without the *p*-hydroxymercuribenzoate; and finally against repeated changes of the phosphate buffer used above.

Incubation

Incubations were performed at 37°C in 8 ml glass vials fitted with screw-caps lined with Teflon. Times of incubation were 0, 4.28, 7.40, 16.28, and 24 hr. Samples were taken from the incubated mixtures at the stated times and transferred to 6 ml Lusteroid ultracentrifuge tubes. The density of these samples was then adjusted to 1.063 with an appropriate NaBr–saline solution and the samples were ultracentrifuged for 24 hr at 114,400 × *g*. The volumes of the samples were selected in such a manner that the amount of ELP floating to the top of the tube would yield only about 15 mg or less of extractable lipid. Larger amounts of ELP form gels which make quantitative recovery of the top fraction material difficult. Upon ultracentrifugation the ELP were concentrated in the top milliliter while the HDL were packed into the bottom milliliter of the tube. These fractions were carefully removed by means of a capillary pipette and the intermediate 4 ml fraction was stored for possible future analysis. The fractions were then placed into 40 ml glass vials (fitted with Teflon-lined screw-caps), flushed with nitrogen, and stored in a freezer at approximately −20°C.

Extraction of the ELP and HDL fractions was performed by a modification of the method of Sperry and Brand (5). The extracted lipids were fractionated into their major constituents (cholesterol esters, glycerides, unesterified cholesterol, and phospholipids) by silicic acid chromatography (6) and quantified by infrared techniques reported earlier (7). Errors and their magnitude (averaging 5–10% for duplicate determinations) incurred in this over-all scheme have been presented in a previous report (1).

The designations of the various incubation mixtures and compositions at 0 hr are shown in Table 1. Lipid contents were calculated from values determined for stock solutions of ELP and HDL prior to ultracentrifugation. In incubation mixtures A and C, the HDL concentration is in the range expected for healthy American women (aged 20–30 yr) (4). The HDL concentration in mixtures B and D is double this value. The two concentrations of ELP used in this investigation are significantly higher than VLDL concentrations encountered in normal subjects. Such concentrations of glyceride-rich VLDL, in the same S_f range of the ELP (primarily in the S_f 20–100 band), are most typically found in human subjects with xanthoma tuberosum (4).

RESULTS

Control Incubations

Table 2 presents data for incubation series E and F and shows the absence of any incubation effect on the lipid composition of ELP and HDL when incubated separately. Percentage recovery of the 0 hr samples of ELP and HDL after ultracentrifugation was 90 and 96% respectively. The lipid composition of the ELP shows an extremely small amount (approximately 0.5% of the total lipid) of cholesterol esters associated with this species. The HDL cholesterol ester content is approximately 34% of the total lipid and is at the lower end of the range of cholesterol ester values determined for human subjects in reported studies (8). These lipoprotein fractions were both put through the same scheme of ultracentrifugal fractionation, after incubation, as was applied to the incubation mixtures containing both ELP and HDL together.

Lipid Transfer between ELP and HDL in Incubation Mixtures A and C

The mole concentrations of the ELP cholesterol esters and glycerides in incubation mixtures A and C are shown as a function of time in Fig. 1. Mole concentrations were calculated using 651 and 885 as the average molecular

TABLE 1 DESIGNATION AND COMPOSITION OF INCUBATION MIXTURES

Incubation Mixture	Lipid Content of Lipoproteins in Incubation Mixture	
	ELP	HDL
	mg/100 ml	
A	780	147
B	780	294
C	390	147
D	390	294
E	780	0
F	0	294

TABLE 2 ABSENCE OF CHANGE IN LIPID CONTENT OF ELP OR HDL WHEN INCUBATED SEPARATELY

Lipid Class	Lipoprotein	Lipid Concentration 0 hr	4.28 hr	7.40 hr	16.28 hr	24 hr
		mg/100 ml				
Cholesterol esters	ELP	5	4	4	4	3
	HDL	98	92	99	90	92
Glycerides	ELP	487	495	516	506	500
	HDL	20	17	18	18	19
Unesterified cholesterol	ELP	27	26	26	24	24
	HDL	16	14	17	18	16
Phospholipids	ELP	181	183	185	184	185
	HDL	151	148	146	147	152

weights of cholesterol esters and glycerides, respectively. Marked and significant increases occurred in the ELP cholesterol esters of both incubation mixtures. They increased in the two mixtures to similar values. The changes in ELP glyceride were all downward with time but are of borderline significance because of the difficulty of detecting relatively small changes in such large amounts of glycerides. The mole concentrations of HDL cholesterol esters and glycerides in these incubation mixtures are shown in Fig. 2. In both incubation mixtures there was a substantial and approximately equal decrease in cholesterol ester contents. The glyceride contents showed a significant and approximately equal increase in both mixtures. After 24 hr the HDL cholesterol ester and glyceride curves approached values which yield mole ratios of approximately 1:1. The ELP and HDL unesterified cholesterol concentrations remained effectively constant during incubation in mixtures A and C. Recovery of total phospholipids appeared to decrease in both mixtures after incubation. The decreases, which were mostly of borderline significance, were primarily confined to the ELP phospholipids. Phospholipids were detected in the intermediate fractions after incubation and although these amounts were difficult to quantify they were of the order of magnitude of the reductions in ELP phospholipids. Further studies are required to establish the behavior of phospholipids in such incubation mixtures.

Lipid Transfer between ELP and HDL in Incubation Mixtures B and D

In this incubation series the ELP concentrations were the same as in the previous series. The HDL concentrations, however, were twice as high. Hence the concentrations of both ELP and HDL were high compared with concentrations of lipoproteins encountered in normal subjects.

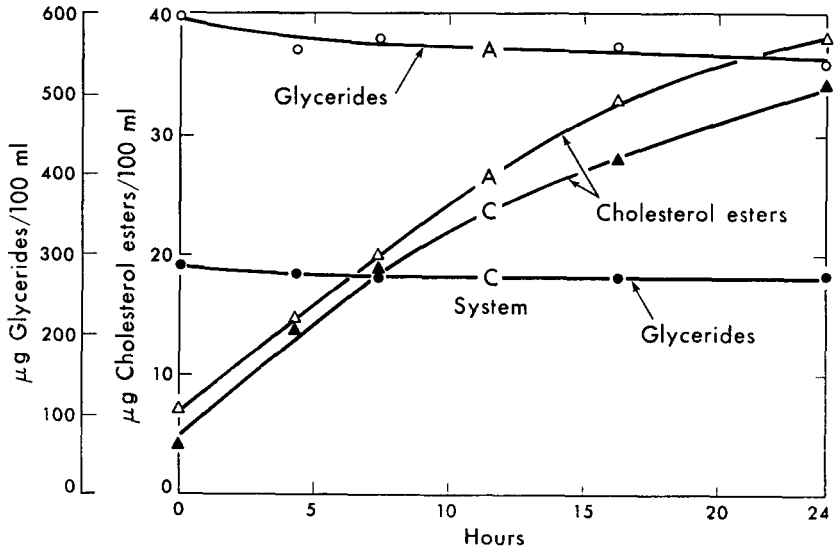

FIG. 1. Changes in ELP cholesterol ester and glyceride mole concentrations during incubation with HDL. Initial concentrations of ELP total lipid were 780 and 390 mg/100 ml in mixtures A and C, respectively. The concentration of HDL total lipid was 147 mg/100 ml in both mixtures.

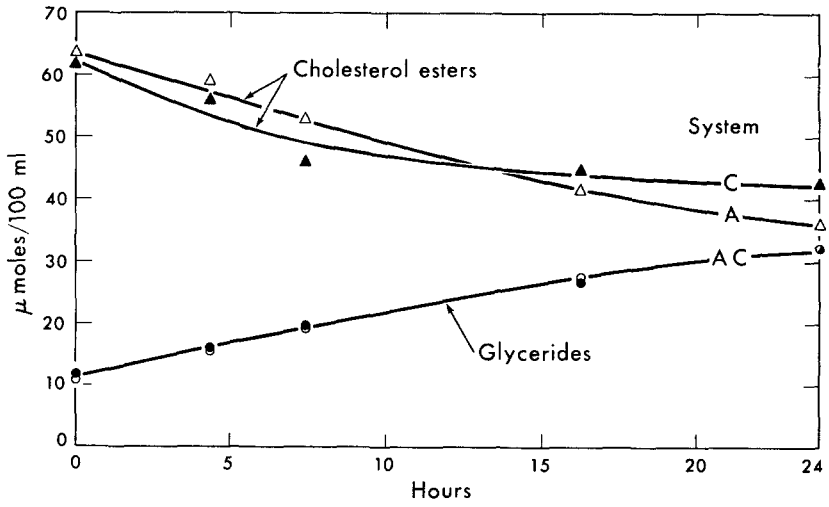

FIG. 2. Changes in HDL cholesterol ester and glyceride mole concentrations during incubation with ELP in mixtures A and C.

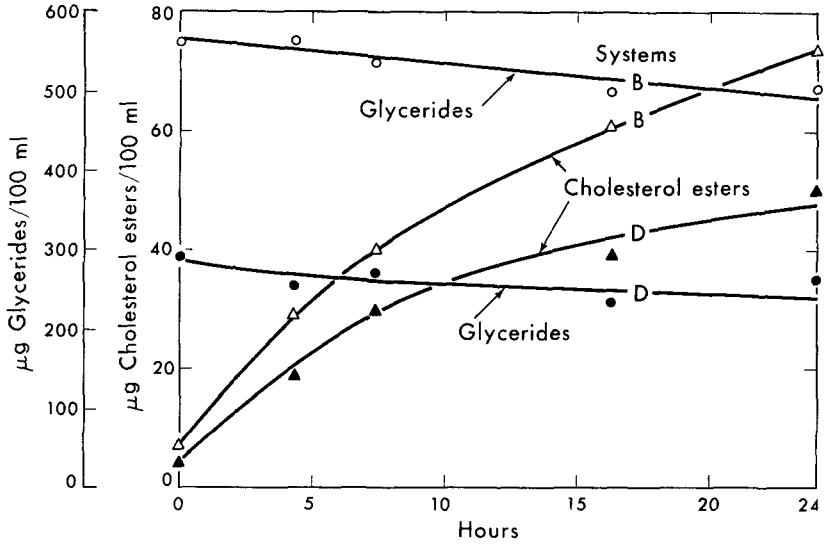

FIG. 3. Changes in ELP cholesterol ester and glyceride mole concentrations during incubation with HDL. Initial concentrations of ELP total lipid were 780 and 390 mg/100 ml in mixtures B and D, respectively. The concentration of HDL total lipid was 294 mg/100 ml in both mixtures.

The mole concentrations of the ELP cholesterol esters and glycerides are presented as a function of time in Fig. 3. There was a marked and significant increase in ELP cholesterol esters in both mixtures. Over the 24 hr incubation the increase in cholesterol esters in mixture B was substantially higher than that observed in D. ELP glyceride concentrations showed a downward trend but again were of borderline significance for the same reasons noted previously for ELP glycerides in A and C.

The mole concentrations of the HDL cholesterol esters and glycerides in the above mixtures are shown in Fig. 4. The cholesterol ester content in mixture B decreased substantially more than in mixture D. The HDL glyceride contents in both mixtures increased, but to a greater extent in mixture B than in D. In incubation mixture B, the HDL cholesterol ester and glyceride concentrations approached the same value and at 24 hr yielded a mole ratio of approximately 1:1. In mixture D the cholesterol ester and glyceride concentrations did not approach comparable values after 24 hr. Table 3 shows the cholesterol ester:glyceride mole ratios in HDL, and the sums of cholesterol ester and glyceride concentrations in mixtures A–D after incubation. The variation in the mole ratio values for the control samples (not incubated, but ultracentrifuged) is due to the variation encountered in the determination of cholesterol esters and glycerides in the HDL fraction for each mixture.

TABLE 3 MOLE RATIOS, CHOLESTEROL ESTERS:GLYCERIDES, IN HDL AFTER INCUBATION OF HDL WITH ELP

Incubation Mixture	Mole Ratio				
	0 hr	4.28 hr	7.4 hr	16.28 hr	24.0 hr
A	5.9:1 (74)*	3.7:1 (75)	2.8:1 (72)	1.5:1 (69)	1.1:1 (69)
B	6.8:1 (164)	3.8:1 (149)	2.8:1 (145)	1.6:1 (144)	1.2:1 (134)
C	5.7:1 (79)	3.4:1 (72)	2.4:1 (66)	1.6:1 (72)	1.3:1 (75)
D	7.0:1 (153)	—† —	3.6:1 (159)	2.5:1 (157)	2.0:1 (158)

* Values in parentheses are sums of mole concentrations (in μmoles/100 ml) of cholesterol esters and glycerides in HDL after incubation with ELP.

† Cholesterol ester sample lost.

The contents of ELP and HDL unesterified cholesterol in both mixtures were unchanged. Recovery of phospholipids decreased in a manner similar to that noted for incubation mixtures A and C. Small amounts of phospholipids were again detected in the intermediate fraction during incubation. However, the changes observed in ELP and HDL phospholipids were not of sufficient consistency or magnitude to establish whether or not phospholipids participate in the observed lipid transfer.

DISCUSSION

These observations extend our earlier findings on lipid transfer in incubated serum (1). The present results show that lipid transfer can occur between isolated HDL and ELP in the absence of serum proteins and enzymes. The lipids primarily involved in the transfer are the HDL cholesterol esters and the ELP glycerides. Further study will be required to clarify the possible involvement of the phospholipids in lipid transfer. Evidence suggesting some phospholipid transfer during incubation studies has been reported by Minari and Zilversmit for dog chylomicrons (9) and by ourselves for human VLDL (transfer to HDL species during incubation) (1).

The data in this report suggest that the transfer reaction approaches equilibrium at some time after 24 hr of incubation. In the course of this partition of lipids the chemical composition of the HDL changes markedly. The sum of the number of moles of cholesterol esters and glycerides on the HDL (see Table 3), however, shows relatively little change, especially in incubation mixtures A, C, and D. Furthermore, the mole ratio of HDL cholesterol esters to glycerides in incubation mixtures A, C, and B appears to approach an integral value of 1:1. The rate at which equilibrium is approached apparently depends on the relative amounts of ELP and HDL lipids rather than on their concentrations in the incubation mixtures. This may be inferred from the observation of comparable rates of equilibration for mixtures B and C which have identical ratios of ELP:HDL lipid but twofold differences in ELP and HDL concentrations. In mixture D, which has the lowest ratio of ELP:HDL lipid, we observed the slowest rate of equilibration. Longer term incubation experiments are currently in progress to establish the period required for full completion of the equilibration which is suggested by the present results.

The approach to a 1:1 ratio between the cholesterol esters and glycerides of the HDL may be simply a consequence of the properties of the equilibration of these two lipids between the ELP and HDL under the conditions of the investigation. It is possible, however, that the occurrence of the 1:1 ratio, together with the relative con-

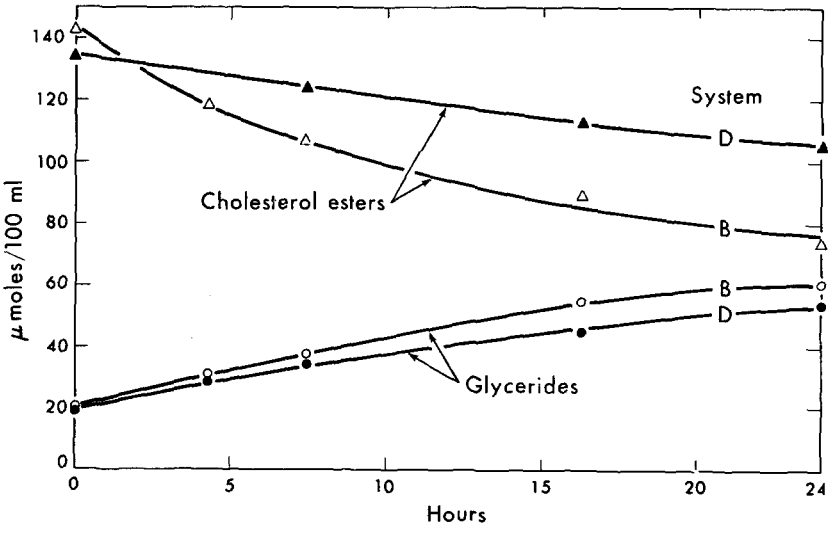

FIG. 4. Changes in HDL cholesterol ester and glyceride mole concentrations during incubation with ELP in mixtures B and D.

stancy of the sum of the mole concentrations of HDL cholesterol esters and glycerides, is indicative of some limiting organization of cholesterol esters and glycerides in the HDL structure. The formation of complexes in mixed monolayer films of lipids and other compounds has frequently been found to occur at simple integral mole ratios for the constitutent components (10, 11). The prolate shape and the estimated axial dimensions which have been reported (12) for the HDL are compatible with a structure having an ordered bimolecular lipid array sandwiched between protein chains. Such a sandwich configuration has been suggested earlier by Vandenheuvel (13).

Evidence suggesting some form of exchange of HDL cholesterol esters with glycerides in vivo has already been reported (14). In these studies, the percentage glyceride content of the HDL lipid was observed to increase with the serum level of VLDL. At VLDL levels above about 800 mg/100 ml, the glyceride content appeared to reach a plateau between 15 and 18% of the total HDL lipid (15). For this glyceride content, the mole ratio of HDL cholesterol esters to glycerides was approximately 2:1. We have not yet observed mole ratios approaching 1:1 for HDL from human subjects (15) or for HDL from incubated serum with added human VLDL (1). It is very possible that the 1:1 mole ratios observed with ELP may be uniquely dependent on the macromolecular and chemical properties of the ELP itself. Clearly, the chemical compositions of the ELP and human VLPL in the same S_f range are grossly different (especially in cholesterol ester content).

The observation of a transfer of HDL cholesterol ester is also interesting in the light of published reports on the absence of exchange of labeled cholesterol esters between serum lipoproteins (16, 17). Such studies have generally been performed at VLDL concentrations lower than were used in our incubations. Nevertheless, some detectable increases of cholesterol ester radioactivity in VLDL have been observed while the HDL cholesterol ester specific activity remained unchanged (17). It has been suggested that exchange of lipids between lipoproteins occurs by diffusion of lipids in a "collison complex" of lipoproteins, where transfer of lipid is determined by energy barriers within the complex (18). Changes in energy barriers for lipid transfer from lipoproteins have been produced by spreading lipoproteins on surfaces (19). The spreading of lipoproteins is believed to lead to some disruption of structure and thereby to changes in lipid associations within them. Hence, in our studies, it is altogether possible that the lipid transfer results effectively from a spreading of the HDL on the relatively large surfaces of ELP which are present in high concentractions in the incubation mixtures.

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