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Research Article

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Interchange of Apolipoproteins between Chylomicrons and High Density Lipoproteins during Alimentary Lipemia in Man

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ABSTRACT Apolipoproteins of the "C" group in human blood plasma, which contain the activator of the lipoprotein lipase-substrate interaction, were found to be transferred specifically from serum to phospholipid-stabilized fat emulsion. Content and distribution of apoprotein activator were measured in healthy men in the postabsorptive state and 4 h after ingestion of meals containing 100 g fat. Content of activator protein in whole serum did not change after ingestion of the fat-rich meals but that contained in triglyceride-rich lipoproteins of density (d) < 1.006 approximately doubled whereas that of high density lipoproteins fell by half. The increased activator content of triglyceride-rich lipoproteins was virtually confined to chylomicrons and its concentration in chylomicron apoprotein was substantially greater than that in very low density lipoproteins. This difference could be ascribed largely to a higher content of C apoproteins in chylomicron protein since both the concentration of C apoproteins and of apoprotein activator were directly proportional to particle diameter while the pattern of fast-migrating C apoproteins in polyacrylamide gels was similar among chylomicrons and subfractions of very low density lipoproteins. Apparent concentration of activator protein was much greater in the high density lipoprotein subfraction of d 1.063–1.125 than in the subfraction of d 1.125–1.21. In the subfraction of d 1.063–1.125, the concentration of activator protein and of fast-migrating C apoproteins in polyacrylamide gels decreased after the fat-rich meal. Concentration of phospholipids in this fraction increased gradually to a peak 43% above the basal value 6 h after the meal. The results obtained demonstrate that high density lipoproteins contribute certain functionally important polar constituents to chylomicrons during alimentary lipemia in man and suggest that they also receive surface constituents from chylomicrons during the course of their metabolism.

INTRODUCTION

Several observations have suggested that the high density lipoproteins of blood plasma (HDL)[1] participate in the transport of triglycerides. During absorption of fat in man and dogs, the content of phospholipids in HDL rises while that of cholesterol changes little (2, 3). This change may depend, in part, upon a transfer of phospholipids from chylomicrons to HDL, such as occurs rapidly when chylomicrons from thoracic duct lymph of dogs are exposed to HDL in vitro (4). The initial step in the catabolism of triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins [VLDL]) is the hydrolysis of their constituent triglycerides by lipoprotein lipase in extrahepatic tissues. Recent studies have shown that formation of the enzyme-substrate complex required for this hydrolysis depends upon the presence of one or more polypeptides ("activator" protein) at the surface of the lipoprotein particles (5–7). From estimates based upon capacity to promote the interaction of phospholipid-stabilized triglyceride emulsions with lipoprotein lipase, it appears that more than half of this activator protein in blood serum of normal human sub-

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[1] *Abbreviations used in this paper:* HDL, high density lipoproteins; R-glu, R-ala, R-ser, apolipoproteins of the C group; VLDL, very low density lipoproteins.

jects in the postabsorptive state is contained in HDL, while the remainder is in VLDL (8). Since this protein is functionally active only in triglyceride-rich lipoproteins and evidently transfers readily from HDL to phospholipid-stabilized fat emulsions (8–10), HDL could provide a source of activator proteins to newly secreted triglyceride-rich lipoproteins. Studies in rats have shown that protein is transferred to lymph chylomicrons when they are exposed to HDL in vitro (11, 12).

In this communication, we report the results of two studies in man that document the participation of HDL in the transport of chylomicrons and indicate that HDL have an important function in the metabolism of exogenous fat.

METHODS

Subjects. Healthy men, age 20–30, reported to the laboratory in the morning after fasting for 12–15 h. After a sample of blood had been obtained, they ingested liquid meals containing 1.5 g fat/kg body weight in the form of cream, safflower oil, or egg yolk (2). Additional samples of blood were taken at intervals for up to 10 h.

Analyses. Lipoprotein fractions were separated from freshly obtained blood serum by sequential flotation at increasing densities in the 40.3 rotor of a Beckman (Beckman Instruments, Inc., Fullerton, Calif.) preparative ultracentrifuge at 12°C (13). Triglyceride-rich lipoproteins ($d < 1.006$) were subfractionated by chromatography on 2% agarose gel (14). Particle diameter of subfractions was estimated from the percentage content by volume of their polar constituents: phospholipids, free cholesterol, and protein (15). Lipids were separated from serum and lipoprotein fractions in ethanol-acetone, 1:1 vol/vol or chloroform-methanol, 2:1 vol/vol and portions were taken for analysis of total cholesterol (16) and lipid P (17). Content of phospholipids was taken as lipid P × 25. Protein was analyzed on washed precipitates from ethanol-acetone extracts by a biuret method (18) or on unextracted lipoprotein fractions by the method of Lowry and associates (19) and expressed as equivalent weight of bovine serum albumin. Turbidity owing to insoluble lipids was removed with diethyl ether before photometry. The ability of serum lipoprotein fractions to promote hydrolysis of triglyceride in phospholipid-stabilized emulsion (Intralipid, Vitrum AB) by lipoprotein lipase from cows' milk was used to estimate content of activator protein as described previously (8) except that 1 mM calcium chloride was added to the incubation mixture. The volume of serum or the weight of protein in lipoprotein fractions required to produce half-maximal activation of lipolytic activity (apparent K_m) was used to estimate concentration of activator protein.[2] Content of polypeptide subunits was evaluated in lipoprotein fractions purified by chromatography in agarose gel (14) or by a second preparative ultracentrifugation. Samples of lipoprotein dissolved in 0.15 M sodium chloride solution containing 0.04% sodium ethylenediaminetetraacetate at pH 7.0 and containing known amounts of protein were mixed with 1,1,3,3-tetramethylurea and subjected to electrophoresis in 7.5% polyacrylamide gel containing 8 M urea at pH 8.9 (21). The gels were fixed in acetic acid and stained with Amidoschwarz 10B. After preliminary destaining with 7% acetic acid, the gels were equilibrated with a standard solution of Amidoschwarz in 7% acetic acid. Optical density of stained gels was determined with a Zeiss PMQ II spectrophotometer (Carl Zeiss, Inc., New York) equipped with a Brinkmann gel scanner module (Brinkmann Instruments, Inc., Westbury, N. Y.).[3] In 4.2 M tetramethylurea, the apoproteins of HDL are separated from lipid in soluble form and migrate quantitatively as known monomeric species (22) in the electrophoretic system. In contrast, only about 3% of the apoprotein of LDL is so solubilized while the remainder is precipitated. A variable fraction of the apoprotein of VLDL (approximately 40%) is precipitated in tetramethylurea. The remainder is in clear solution and migrates quantitatively (21). The patterns obtained in tetramethylurea are similar to those observed after preliminary delipidation of HDL and VLDL.

RESULTS

Concentration of activator protein in subfractions of triglyceride-rich lipoproteins. Concentration of activator protein was assayed in samples obtained in the postabsorptive state and 4 h after ingestion of 1.5 g cream fat/kg body weight. As shown in Table I, the quantity of protein required to produce half of the maximal rate of hydrolysis of triglycerides was related inversely to particle diameter. No systematic difference was observed between the concentration of activator protein in fractions of comparable diameter obtained before and after the fat-rich meal. In two subjects, sufficient protein was obtained to evaluate content of polypeptide subunits in subfractions obtained after the fat-rich meal. In each subject, the pattern of the fast-migrating group of bands was similar in particles of all sizes but the percentage content of tetramethylurea-soluble protein was related directly to particle diameter (Fig. 1).

Effects of fat-rich meals upon content of activator protein in serum and lipoprotein fractions. When phospholipid-stabilized triglyceride emulsion was exposed to serum freed of VLDL by ultracentrifugal flotation, it acquired a group of polypeptides whose mobilities in poly-

[2] The major activator of the interaction of emulsified triglycerides with lipoprotein lipase of cows' milk is a protein with carboxyl-terminal glutamic acid (R-glu) (5, 20). At least two other apolipoproteins of the C group with carboxyl-terminal alanine (R-ala) and carboxyl-terminal serine (R-ser) inhibit the activation by R-glu (references 5 and 20 and Havel, R. J., and V. G. Shore, unpublished data). In the proportions of R-glu, R-ala, and R-ser found in triglyceride-rich lipoproteins of normolipidemic subjects of about 1:3:1 (Shore, V. G., unpublished data), inhibition of the activator property of an amount of R-glu yielding half-maximal activation in our assay system is less than 15%. Although the proportion of these three polypeptides probably varies to some extent and their chromogenicity in the Lowry method (19) for protein is related to their amino acid composition, we consider that this assay is useful for the detection of substantial changes in the concentration of activator protein.

[3] Optical density of authentic R-glu or R-ala subjected to electrophoresis was linearly related to concentration over a range of 2–22 μg.

TABLE I

*Activator Property of Total Protein of Chylomicrons and Subfractions of VLDL**

Nutritional state and subject	Particle diameter (Å)			
	>800	550–650	400–500	300–400
Postabsorptive				
G. C.	2.8	4.0	3.6	7.2
N. S.	3.0	5.6	11	25
E. J.	3.4	4.5	6.2	10
Mean	3.1	4.7	7.0	14
Fat fed				
G. S.	1.3	3.4	5.9	13
R. W.	4.3	7.7	20	33
J. P.	1.8	3.3	5.3	14
Mean	2.5	4.8	10	20

* Micrograms protein per milliliter medium at apparent K_m.

acrylamide gel were closely similar to those of VLDL from the same subject (Fig. 2). Such emulsions had maximal rates of hydrolysis of constituent triglycerides when incubated with lipoprotein lipase from cows' milk in the standard incubation system without further addition of serum or active lipoprotein fraction. To test the possibility that such transfer occurs when chylomicrons enter the blood in vivo, content of activator protein in serum $d < 1.006$ lipoproteins and the remaining serum proteins of $d > 1.006$ was measured in seven subjects before and 4 h after ingestion of the standard meal of cream fat. Results, summarized in Fig. 3, indicate that content of activator protein in serum remained steady or decreased slightly while that in triglyceride-rich lipoproteins ($d < 1.006$) uniformly increased (mean change + 113%, SD 32) and that in residual serum, containing other serum lipoproteins, uniformly fell (mean change —49%, SD 16). Since activator protein in the latter fraction is confined to HDL (8), two subfractions of this lipoprotein class were separated from serum before and 4 h after the standard fat-rich meal in six additional subjects. Most of this activity was found in the HDL_s subclass ($d = 1.063$–1.125) while the HDL_s subclass ($d = 1.125$–1.21) contained so little that its content of activator protein could usually not be quantified. Concentration of activator protein in HDL_s fell uniformly after the fat-rich meal (Table II). This was accompanied by sub-

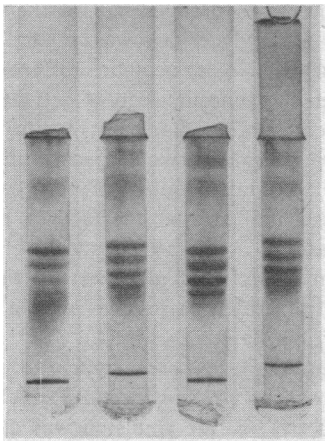


FIGURE 1 Polyacrylamide gel electrophoretic patterns of tetramethylurea-soluble apoproteins of chylomicrons and subfractions of VLDL separated from blood serum of a healthy man 4 h after ingestion of a meal containing 100 g of cream fat. From left: chylomicrons (diameter > 1000 Å), followed by subfractions of VLDL with approximate mean diameters of 700, 450, and 350 Å. Percentage content of protein soluble in tetramethylurea, estimated by the Lowry method (19), was 89% in the chylomicrons and 79, 60, and 47% respectively in the VLDL fractions of decreasing particle diameter. The slowest of the fast-migrating group of bands comigrates with authentic R-glu and the three bands below it comigrate with authentic subspecies of R-ala.

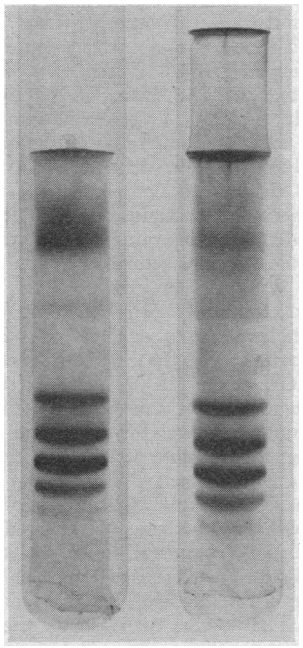


FIGURE 2 Polyacrylamide gel electrophoretic patterns of tetramethylurea-soluble apoprotein of VLDL from a healthy man (left) and of protein adsorbed to particles of triglyceride emulsified with lecithin (Intralipid) after exposure to $d > 1.006$ fraction of serum (right). In the latter case, 20 ml of the serum-fraction was incubated with 2 ml of 10% Intralipid at 37°C for 30 min. The mixture was layered under 0.15 M sodium chloride solution containing 0.04% sodium ethylenediaminetetraacetate and centrifuged for 2×10^6 *g*-min. The supernatant emulsified particles were collected, resuspended in the saline solution, and the centrifugation was repeated four times to remove nonadsorbed proteins. In each case, approximately 115 μg of protein was applied to the surface of the running gel.

stantially reduced content of fast-migrating bands corresponding to the major activator polypeptide, R-glu, and subspecies of the other fast-migrating polypeptide, R-ala, as determined by electrophoresis of HDL$_3$ containing equal amounts of protein (Fig. 4). Fast-migrating bands were barely detectable in HDL$_3$ and no difference was apparent between samples obtained before and after the meal.

Effects of fat-rich meals upon content of lipids and protein in serum lipoproteins. Two subjects were given single meals containing, respectively, cream fat, safflower oil, or egg yolk at intervals of 1 wk. Samples of serum were subjected to quantitative preparative ultracentrifugation at *d* 1.006, 1063, 1.125, and 1.21 to separate VLDL plus chylomicrons, LDL, HDL$_2$, and HDL$_3$. Results are shown in Fig. 5. Content of total cholesterol, phospholipids, and protein rose and fell over comparable intervals of time in VLDL with maximal values occurring 4.5 h after the meal. The relative increases in these constituents were roughly comparable with their content in chylomicrons (23). Content of phospholipids and protein rose in HDL$_3$ in every case, but more slowly, with maximal values 6 h after the meal. The maximal increase of phospholipids in HDL$_3$ was 43±14% (mean±SD) and that of protein was 24±6% above the basal level. Content of phospholipids but not cholesterol also increased in HDL$_3$ and was highest 10 h after the meal (maximal increase 15 +7% above the basal level). Content of protein could not be measured accurately in HDL$_3$ because of contamination with albumin and other serum proteins. In LDL, the only consistent change was a fall in total cholesterol (maximal reduction 6.3 + 4.6% below the

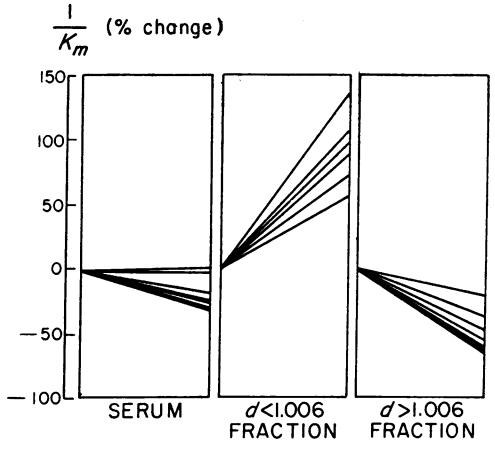


FIGURE 3 Content of activator protein in whole serum from seven healthy men and in two ultracentrifugal fractions separated at *d* 1.006 in the postabsorptive state and approximately 4 h after ingestion of 100 g of cream fat. The reciprocal of the quantity of serum required to give half of the maximal rate of hydrolysis was calculated to give a value directly related to content of activator protein. The lines indicate the percentage change observed for each subject after ingestion of the fat-rich meal. Mean values ±SD are given in the text.

TABLE II
*Activator Property of Total Protein of High Density (1.063–1.125) Lipoproteins**

Subject	Post-absorptive	Fat fed
J. A.	28	>55
S. A.	38	63
J. P.	38	63
J. G.	20	>67
P. Q.	30	>100
M. B.	27	53

* Micrograms protein per milliliter medium at apparent K_m.

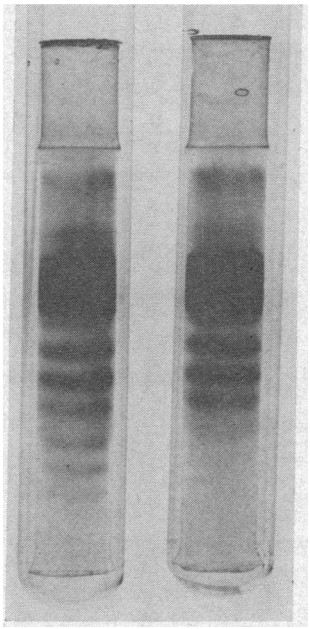
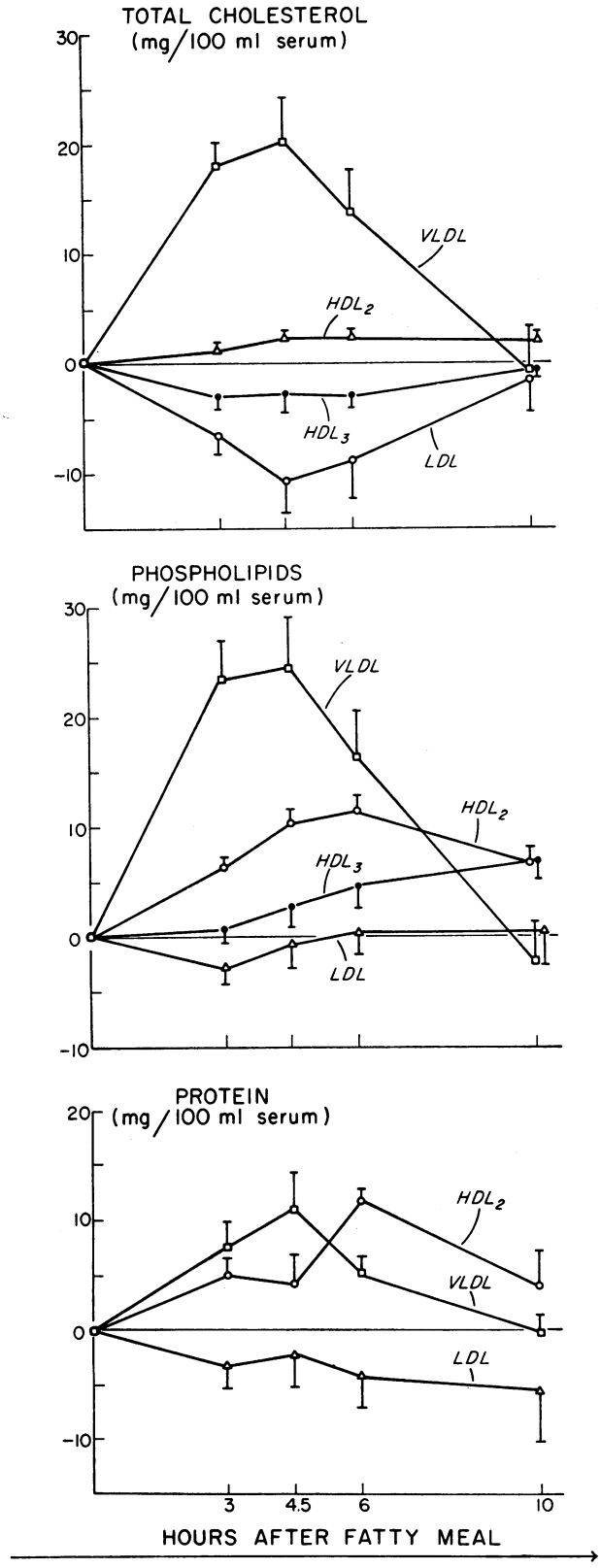


FIGURE 4 Polyacrylamide gel electrophoretic patterns of tetramethylurea-soluble proteins of HDL$_3$ obtained from a healthy man (J. G.) in the postabsorptive state (left) and 4 h after ingestion of 100 g of cream fat (right). In each case, an amount of lipoprotein containing 246 μg protein was applied to the surface of the running gel (the gel was overloaded to bring out the fast-migrating C apoproteins). Based upon results obtained with authentic polypeptides, the principal band in these patterns corresponds to R-threonine and the three prominent bands below it all correspond to R-glutamine when the gels contain reducing agents (21). The first of the three bands which migrate most rapidly corresponds to R-glu and the other two to subspecies of R-ala. The other bands were not identified. The scans of optical density of these gels showed approximately three fold reduction of the three fastest-migrating bands in the sample taken after the fat-rich meal. The activator content of these lipoproteins is shown in Table II.



basal level 4.5 h after the meal). No systematic differences were observed for these changes in content of lipids and protein of lipoprotein fractions among the three types of fat fed to these two subjects.

DISCUSSION

The results of this study provide strong evidence that activator protein is transferred from HDL_2 to chylomicrons during alimentary lipemia in healthy young men. The consistent, approximately twofold decrease in the apparent concentration of activator protein in HDL_2 (Table II) together with the large reduction of the content of the several fast-migrating species of the apoprotein of HDL_2 on polyacrylamide gels (Fig. 4), which include the active polypeptide, R-glu, as well as the subspecies of R-ala indicates that a substantial fraction of these C apoproteins leave HDL_2 during alimentary lipemia. Since no evidence was obtained for entry of additional activator protein into the blood (Fig. 3) it is reasonable to conclude that these proteins are transferred to chylomicrons in a manner analogous to their transfer to phospholipid-stabilized particles of emulsified fat.

The amount of activator protein in the total apoprotein and the percentage content of tetramethylurea-soluble protein were both greater in chylomicrons than in VLDL and, in the latter, they were directly related to particle size. No gross differences in the relative amounts of fast-migrating species were observed in polyacrylamide gel patterns of proteins from chylomicrons or subfractions of VLDL. Evidently, at least part of the difference in activator content of the subfractions is attributable to increasing percentage content of "B" apoprotein in the total protein with decreasing particle size. Very recently, similar estimates of content of "B" apoprotein in subfractions of VLDL have been obtained by gel filtration of urea-soluble apoproteins of VLDL from two hyperlipemic subjects (24). Content of activator protein in the subfractions of VLDL changed little in our subjects after the fat-rich meal and no gross differences were observed in the polyacrylamide gel patterns of these subfractions. This suggests that the fast-migrating polypeptides are bound less tightly to HDL_2 than to VLDL. It is notable that a strong inverse relationship exists between the concentrations of HDL_2 and VLDL (25). Thus, the pattern and extent of transfer of the fast-migrating polypeptides during alimentary lipemia may differ in subjects with endogenous hyperlipemia from that observed in the present study.

FIGURE 5 Mean changes ±SEM for constituents of four lipoprotein fractions separated from serum obtained at intervals after ingestion of three separate meals containing 100 g of fat in each of two subjects. Each meal contained fat from a different source: cows' milk, safflower seed oil, and hens' eggs.

An analogous transfer of fast-migrating polypeptides may occur when VLDL are secreted from the liver into the blood of rats (26). Nascent VLDL isolated from a Golgi apparatus-rich fraction of rat liver contain about two-thirds of the amount of protein found in VLDL separated from blood serum. Content of fast-migrating polypeptides and of activator protein in nascent VLDL are substantially lower than those of serum VLDL. After the nascent VLDL are mixed with HDL from rat serum and separated from them by ultracentrifugation, content of protein increases. The polyacrylamide gel pattern and content of activator protein then closely approximate those of serum VLDL. The increased content of protein is accompanied by a reciprocal reduction in content of phospholipids. These two sets of observations in humans and rats, together with data obtained previously (2–4, 11, 12), suggest that reciprocal transfers of phospholipids and fast-migrating polypeptides occur after triglyceride-rich lipoproteins are secreted from intestinal mucosa and liver of several mammalian species. Since both phospholipids and protein appear to occupy the surface of these lipoproteins (15), transfer of phospholipid from triglyceride-rich lipoproteins to HDL might facilitate the reciprocal transfer of a specific group of polypeptides from HDL. Such a phenomenon could explain our observation that the gradual rise in content of phospholipids in HDL during alimentary lipemia occurs chiefly in HDL_2 since this is the site from which fast-migrating polypeptides are transferred to chylomicrons. The increased content of protein in HDL_2 during alimentary lipemia is in apparent conflict with this interpretation. Possibly, altered structure of HDL_2 associated with its substantially changed chemical composition (43% increase in phospholipid content) leads to transfer of protein from other sites, such as HDL_3. Participation in such an interchange is suggested by the subsequent rise in content of phospholipid in HDL_3 when that in HDL_2 fell 10 h after the fat-rich meal (Fig. 5). Mean content of phopholipids in HDL_2 plus HDL_3 was 16 mg/100 ml higher than the fasting value 10 h after the meal when that of phospholipids in trglyceride-rich lipoproteins had fallen to the basal level (Fig. 5). This suggests that, in part, transfer of phospholipid may accompany the return of polypeptides to HDL once triglycerides have been removed through the action of lipoprotein lipase. This could serve to remove "excess" surface material from chylomicron "remnants" (27–29). As observed previously (2), no change in content of phospholipids or protein occurred in LDL during alimentary lipemia. The fall in content of cholesterol in LDL was not observed earlier. It could reflect transfer of both free (4) and esterified (30) cholesterol respectively between the surface and "core" of chylomicrons and LDL since the time-course of reduction of total cholesterol in LDL mirrored its increase in triglyceride-rich lipoproteins (Fig. 5).

The group of polypeptides which transfers from HDL to triglyceride-rich lipoproteins appears to have specific affinity for interfacially oriented monolayers of phospholipids. The polyacrylamide gel pattern of polypeptides which are acquired by the phospholipid-stabilized fat emulsion, Intralipid, was strikingly similar to that of those proteins of VLDL that migrate in such gels. Our observations are therefore consistent with the concept that these "C" apoproteins constitute a functional entity (31). The site of origin of these polypeptides evidently includes the liver, since nascent VLDL isolated from a Golgi apparatus-rich fraction of rat liver contain a small amount of fast-migrating polypeptides and of activator protein (26) and labeled lysine is incorporated into fast-migrating polypeptides of VLDL secreted from perfused rat liver (32). In contrast, the intestinal mucosa does not appear to secrete fast-migrating polypeptides since they do not become labeled in newly secreted triglyceride-rich lipoproteins when rat intestine is perfused similarly (32) and we observed no increase in content of activator protein in blood serum during alimentary lipemia in man. The results of the present study, together with the slow fractional catabolic rate of fast-migrating polypeptides in lipoproteins of human blood plasma (33), suggest that small amounts of these polypeptides are secreted continuously from the liver in VLDL and that they then recycle repeatedly between triglyceride-rich lipoproteins and HDL before they undergo catabolism (34).

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