LIPID TRANSFER BY HIGH DENSITY LIPOPROTEINS OF HUMAN SERUM IN VITRO[1]

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High density lipoproteins (HDL) constitute one of the major classes of lipid transport in human serum. The characteristic lipid composition of this lipoprotein class, isolated by ultracentrifugal techniques, has been well established (Lindgren et al., 1956; Bragdon et al., 1956). Recent studies show that the HDL class may take up specific lipids and thereby alter its chemical composition. Thus, HDL from sera of individuals ingesting a fat load show a significant increase in glyceride content (Nichols et al., 1962). This increase in HDL-glyceride is associated with a marked increase in serum level of the glyceride-rich very low density lipoproteins (VLDL) of density ≤ 1.006 g/ml. Subsequent studies on normal individuals without fat loading show a high correlation between HDL-glyceride content and the total serum glyceride level (Lindgren et al., 1964). These observations suggest that a glyceride transfer process may occur when HDL are exposed to elevated levels of VLDL. HDL species have been shown by Ashworth et al. (1963) to bind glyceride when exposed to glyceride dispersed on Celite 545 (Johns-Manville). In this paper we report on preliminary experiments showing the existence of a lipid transfer process, involving HDL species, which occurs during the incubation of VLDL or other glyceride-rich macromolecules with human serum. Furthermore, we find that during the binding of glyceride by HDL there occurs a marked displacement of HDL-cholesteryl esters.

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Experimental - The standard incubation system consisted of a 5 ml aliquot of serum to which was added a 1 ml aliquot of a saline solution of either glyceride-rich lipoproteins, human VLDL and chicken egg yolk lipoprotein (EYL), or a glyceride emulsion (Ediol)[1]. Sera were drawn from 4 healthy subjects (A, B, C, and D) with low serum levels of VLDL. Subjects A, B, and C were females (ages 40-45) and subject D was a male, 27 years. The initial concentrations of total HDL lipid in the incubation mixtures with sera A, B, C, and D were 151, 140, 149, and 102 mg/100 ml, respectively. Thus, the concentrations of HDL in the mixtures containing sera A, B and C were approximately equivalent. The human VLDL were isolated ultracentrifugally from sera of healthy individuals exhibiting high levels of these species. EYL were isolated ultracentrifugally in saline from fresh chicken egg yolks (Nichols et al., 1954). Following incubation for 16 hours (at 37° C and with mild shaking) the mixtures were ultracentrifugally fractionated to obtain the major lipoprotein classes. Lipid analysis of isolated lipoprotein fractions consisted of extraction, silicic acid column chromatography, and infrared spectrophotometry (Freeman et al., 1963). Samples were studied in duplicate and the error in the overall preparative and analytic scheme ranged between 2 - 5% for the glyceride and cholesteryl ester values reported.

Results and Discussion - The data presented in Table I show that with elevated levels of VLDL-glyceride in the incubation mixture there is definite uptake of glyceride by the HDL. The uptake of glyceride does not appear to be linear with respect to the VLDL-glyceride concentration. This would suggest a saturation of HDL by glyceride at the higher levels of VLDL-glyceride in the medium. The concentration of HDL-cholesteryl esters is also significantly influenced by the level of VLDL-glyceride in the medium. At the lower levels of VLDL-glyceride, the cholesteryl ester concentration either increases or remains constant. At higher levels

[1] Oral fat emulsion, Schenlabs Pharmaceuticals, Inc., New York, N.Y.

TABLE I

Effect on HDL Lipids of Incubation of Serum with VLDL

Serum	VLDL-Glyceride Concentration (Mg/100 ml)	HDL Lipid Concentration (Mg/100 ml)			
		Glycerides		Cholesteryl Esters	
		Control	Incubated	Control	Incubated
A	16	5	6	64	76
A	126	5	21	64	64
A	222	5	28	64	58
A	429	5	33	64	49
B	346	13	31	57	53
C	460	8	38	58	36

of VLDL-glyceride there is marked reduction of HDL-cholesteryl ester concentrations. The increase in HDL-cholesteryl esters, at low VLDL-glyceride concentrations, is a result of the activity of a serum fatty acid transferase whereby lipoprotein-attached unesterified cholesterol is esterified with fatty acids derived from lipoprotein phospholipid (Sperry et al., 1955; Glomset, 1963). The resulting cholesteryl esters are attached to the serum lipoproteins, such as the HDL. However, with increasing levels of VLDL in the mixture, the data indicate that the newly-formed as well as some of the previously-existing cholesteryl esters of the HDL are removed. When the transferase is inhibited by p-hydroxymercuribenzoate no increases in HDL-cholesteryl esters are observed and the reduction in HDL-cholesteryl esters is directly a function of VLDL-glyceride level (unpublished observations). Current studies also show that the transfer of HDL-cholesteryl esters is primarily to the VLDL (unpublished observations).

The effect of a very different VLDL-type lipoprotein, EYL, is shown in Table II. Very high EYL-glyceride levels were used initially in order to establish the effect. These data show lipid changes in the HDL very analogous to those found when using human VLDL. The final HDL-glyceride values again suggest a saturation effect. The reduction in HDL-cholesteryl esters is appreciably greater than that observed using human VLDL.

TABLE II

Effect on HDL Lipids of Incubation of Serum with EYL

Serum	EYL-Glyceride Concentration (Mg/100 ml)	HDL Lipid Concentration (Mg/100 ml)			
		Glycerides		Cholesteryl Esters	
		Control	Incubated	Control	Incubated
B	701	13	40	56	26
D	770	14	36	42	16

TABLE III

Effect on HDL Lipids of Incubation of Serum with Ediol

Serum	Ediol-Glyceride Concentration (Mg/100 ml)	HDL Lipid Concentration (Mg/100 ml)			
		Glycerides		Cholesteryl Esters	
		Control	Incubated	Control	Incubated
A	488	5	17	64	60
A	733	5	21	64	52

The data in Table III show that the effect of incubation with Ediol is qualitatively comparable but of relatively lesser magnitude than observed with VLDL or EYL as glyceride sources.

These data show the existence of a lipid transfer property of human HDL resulting from the exposure of HDL to elevated levels of glycerides in macromolecules from diverse sources. Since serum contains other lipoprotein species as well as fatty acid transferase activity, both of which may affect the degree and character of lipid transfer, studies are in progress on lipid transfer between isolated lipoprotein species. The studies reported here open up possibilities for the investigation of lipoprotein structure by the attachment and removal of lipids, as well as the investigation of the implications, if any, of such lipid transfer to lipid metabolism.

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