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Apparent Inhibition of Cholesteryl Esters Exchange by High Density Lipoproteins

J. M. Ruiz-Albusac and A. Montes *

Departamento de Bioquímica
Facultad de Medicina. Universidad Complutense
28040 Madrid (Spain)

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The kinetics of the exchange of Cholesteryl esters between low density lipoproteins (LDL) and high density lipoproteins (HDL) stimulated by lipoprotein depleted plasma has been studied *in vitro*. The results indicate that the exchange is inhibited with the increase of HDL present in the assay, although the limiting factor is not the absolute concentration of HDL, since in a simultaneous LDL increase, the exchange augments proportionally to the total cholesteryl esters pool. Implications regarding overall metabolism of body cholesterol are discussed.

Key words: Cholesterol, Lipoproteins.

Cholesterol is an essential structural component of all cell membranes. Most tissues are unable to catabolize cholesterol or excrete it from the body. The transport of cholesterol from such tissues to sites of catabolism (liver, adrenal cortex and gonads) and excretion (liver, skin and intestine) may be a function of plasma high density lipoproteins (HDL). The release of cholesterol from cells occurs in the unesterified form (10, 17) and it has been suggested that the esterification of cholesterol in HDL by lecithin: cholesterol acyl transferase (LCAT) may facilitate the uptake of further cholesterol molecules (7). The importance of HDL as an acceptor of «excess» free cholesterol liberated during triglyceride depletion in very low density lipoproteins (VLDL) and chylomicrons has also been emphasized (6). The cholesteryl esters (CE) thus formed may be transferred HDL to remnants, to be eventually removed by the receptor mediated mechanism of extrahepatic cells (13). This scheme is in accordance with the hypothesis that most, if not all, of the CE of human plasma lipoproteins are formed by the action of LCAT (15). Since VLDL, chylomicrons and low density lipoproteins (LDL) lipids are themselves poor substrates for LCAT (2), their CE must be transferred from HDL, which is the best substrate for the enzyme.

* To whom all correspondence should be addressed: Departamento de Fisiología. Facultad de Farmacia. Universidad de Alcalá de Henares. Alcalá de Henares/Madrid (Spain).

The mechanism of CE transfer among lipoproteins has been the subject of intense debate, since these highly hydrophobic lipids in the core of the lipoprotein particle are thought to exchange through the surrounding media only slowly or not at all. However, significant evidence has now demonstrated exchange or net transfer of CE between different lipoprotein classes. CE exchange between VLDL and LDL or between HDL and other lipoprotein is stimulated by a protein present in lipoprotein depleted plasma (LDP) (16, 20). This protein would bind to HDL to such an extent that exchange of CE between lipoproteins is diminished at high HDL concentrations (3).

In the present report we study the kinetics of CE exchange between LDL and HDL stimulated by lipoprotein depleted plasma, and its inhibition by HDL.

Materials and Methods

Isolation of lipoproteins. Human LDL and HDL were isolated by sequential ultracentrifugation at the appropriate density range (11, 12), LDL between 1.006-1.050 g/ml, and HDL between 1.063-1.21 g/ml. In order to prepare LDP, human plasma was brought to a density of 1.25 g/ml, and ultracentrifuged in a Beckman 50 Ti rotor, at 140,000 *g* for 40 h, at 14°C. The top fraction was discharged and the bottom fraction (LDP) was dialyzed against 0.1 M sodium phosphate buffer, pH 7,4, with 0.15 M NaCl, 1 mM Na_2EDTA. LDL and HDL were dialyzed against the same buffer after isolation.

Incubation of lipoproteins. To obtain labelled LDL, a filter paper disc impregnated with ^{3}H-cholesterol (The Radiochemical Center, 43 Ci/nmol) was incubated with fresh human plasma at 37°C for 48 h. After incubation, LDL was isolated as before and incubated with a 20-fold excess of human HDL for 4-6 h in order to remove labelled unesterified cholesterol. The mixture was then ultracentrifuged at a density of 1.050 to reisolate the LDL. The final product had less than 5 % of the radioactivity in free cholesterol.

^{3}H-CE transfer assay was performed as indicated by PATTNAIK *et al.* (16). Labelled LDL was incubated with cold HDL for 3 h with different amounts of LDP. All the assays were carried out at 37°C in 0.1 M phosphate buffer, pH 7.4 with a final volume of 1 ml. At the end of the incubation period 2 ml of 6 % bovine serum albumin (fraction V, Sigma) in 0.1 M phosphate buffer, pH 7.4, was added to each vial, and LDL was precipitated with heparin and $MnCl_2$ (5). After centrifugation (6,000 *g* for 15 min), radioactivity was determined in an aliquot of supernatant with toluene-Triton X-100 scintillation fluid.

Calculations. The catalyzed transfer of CE from LDL to HDL during incubation was expressed as exchange activity and calculated as % Kt (20). The rate of exchange of CE from LDL to HDL was calculated using the formula (4)

$$F_A = \frac{DPM_{(HDL)}}{\int_0^3 (S_{LDL} - S_{HDL})\, dt}$$

F_A (apparent flux), represents the unidirectional flux of CE (μg/h/incubation) from LDL to HDL or viceversa, given that there is no net change of CE mass in any fraction at the end of incubation. S_{LDL} and S_{HDL} represent the specific activities of esterified cholesterol in donor and receptor fractions at time t. The rates of exchange have been calculated from values of zero hours and three hours, assuming that the exchange is lineal during this period of time.

To calculate the total flux (F_T) taking place in the assay, we assume that the

rate of exchange of CE within HDL or LDL particles is the same as the rate of exchange from LDL to HDL. If this is so, the exchange among HDL or LDL CE molecules is F_A(HDL)/(LDL) and F_A(LDL)/(HDL) respectively, and

$$F_T = F_A \frac{(HDL) + (LDL)^2}{(HDL) \times (LDL)}$$

(LDL) and (HDL) represents the CE concentrations of each fraction.

Other methods. Lipoprotein cholesterol was extracted by partitioning between 43 % aqueous ethanol and hexane, and the unesterified and ester forms were separated by thin layer chromatography on silica gel H with hexane/diethyl ether/acetic acid (50:50:1, v:v:v). Total cholesterol was measured by the method of ZAK *et al.* (19) after ABELL *et al.* saponification (1). Total protein was measured by the biuret method (9).

Results

In order to confirm that there is no spontaneous transfer of CE between lipoprotein fractions (8), preliminary experiments were carried out in which LDL was incubated for 3 h with HDL labelled in its CE. At the end of this period no change in the amount of free or esterified cholesterol was found in any fraction, nor was there labelled CE in LDL. When the labelled fraction was LDL, only 2 % of the radioactivity was recovered in HDL-CE.

The effect of HDL in the CE exchange stimulated by LDP is shown in figs. 1 and 2. When increasing amounts of human LDP were incubated with two different HDL concentrations and the same ^{3}H-LDL, the transfer of ^{3}H-CE from LDL to HDL was higher with the lower HDL, and this effect was particularly important at the highest LDP concentration tested (fig. 1). To emphasize the fact

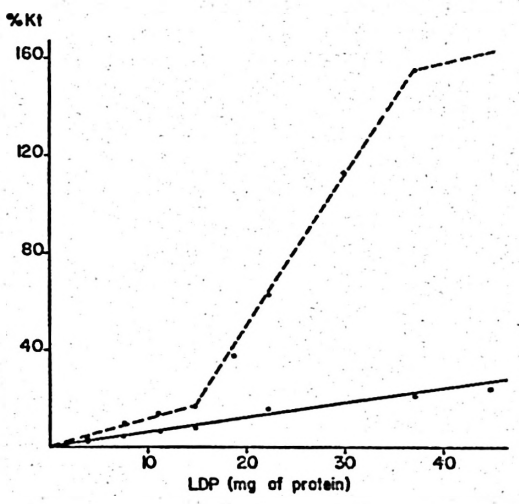

Fig. 1. *CE transfer from LDL to HDL stimulated by LDP.*
Human LDL, labelled in its CE, was incubated with 5 times (----) or 13 times (——) excess of human HDL (measured in total cholesterol) and increasing quantities of LDP, for 3 h at 37°C. At the end of this period, the LDL was precipitated and the ^{3}H-CE transferred to HDL were calculated as % kt.

that the amount of HDL in the assay interfered with the transfer of ^{3}H-CE from LDL, ^{3}H-LDL was incubated with increasing quantities of HDL and a fixed amount of LDP (18.5 mg of protein). Figure 2 shows that HDL produced a linear decrease in the transfer of CE between both lipoproteins. In both experiments, total and esterified cholesterol were measured in HDL and LDL at the end of each incubation (data not shown); in either case there was no difference with the initial contents of the fraction, which means that the transfer of radioactivity represents exchange of CE and not net transfer (20).

The inhibition of CE exchange can be either directly caused by HDL or a consequence of the changes in the relative contributions of HDL and LDL to the total CE pool in the assay. In order to

Table I. *Cholesteryl ester flux stimulated by LDP.*
Each incubation contains 14 μg of cholesteryl esters in LDL and 79 μg in HDL.

LDP mg of protein	Specific activity at 3 h LDL	HDL	Apparent flux μg/h/Inc.	Total flux μg/h/Inc.
0.0	7143	0	—	—
3.7	6690	80	0.1024	0.8008
7.4	6221	163	0.2173	1.6993
11.1	5997	203	0.2756	2.1552
14.8	5782	241	0.3338	2.6103
18.5	4701	433	0.6657	5.2058
22.2	3312	679	1.2190	9.5327
29.6	2113	891	1.8712	14.6329
37.0	1305	1035	2.4498	19.1576
44.4	1221	1049	2.5189	19.6950

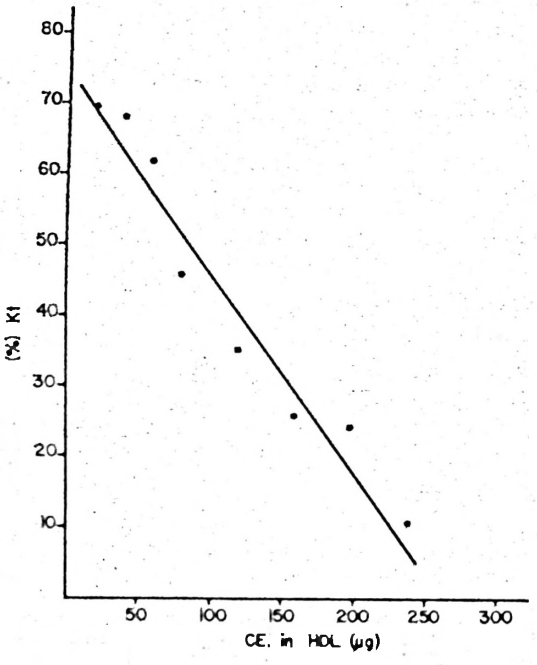

Fig. 2. *Effect of HDL concentration on LDL-CE transfer.*
Human LDL containing 14 μg of ^{3}H-CE was incubated with 18.5 mg of LDP protein and increasing quantities of human HDL, for 3 h at 37°C. At the end of this period, the LDL was precipitated and the ^{3}H-CE transferred to HDL were calculated as % kt.

differenciate both possibilities, experiments with different concentrations of ^{3}H-LDL and HDL were carried out, and both the apparent and total flux were calculated. Tables I and II, and fig. 3 show that apparent and total fluxes can

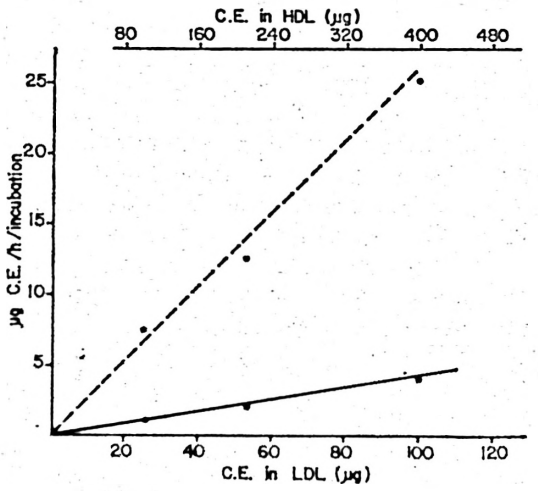

Fig. 3. *CE flux among LDL and HDL at increasing concentrations of both lipoproteins.* Increasing quantities of ^{3}H-LDL and HDL were incubated, at a fixed LDL:HDL CE ratio of 1:4, with 7.4 mg of LDP protein at 37°C. At the end of the incubation period (3 h), apparent (———) and total (- - - -) CE fluxes were calculated as indicated in methods.

Table II. *Cholesteryl ester flux stimulated by LDP.*
Each incubation contains 18 mg of LDP protein and 14 μg of cholesteryl esters in LDL.

HDL esterified cholesterol (μg)	Specific activity LDL	Specific activity HDL	Apparent flux μg/h/inc.	Total flux μg/h/inc.
0.00	7143	0	—	—
19.75	3565	2536	1.3625	5.6129
39.50	3625	1247	1.1495	5.9496
59.25	3869	773	0.9948	6.4348
79.00	4535	462	0.7235	5.6578
118.50	5048	247	0.5457	5.7748
158.00	5520	144	0.4034	5.3952
197.50	5597	110	0.3808	6.1606
237.00	6413	43	0.1681	3.1918

vary in different ways: When the amounts of LDL and HDL were fixed (Table I), the apparent and total flux increased linearly with the increase in LDP protein; However, when a fixed amount of LDL was incubated with increasing quantities of HDL and the same LDP (Table II), the apparent flux decreased linearly, but the total flux was the same for any HDL concentration. Finally, when both LDL and HDL were increased in the same proportion, so that the LDL:HDL ratio was fixed, with a constant amount of LDP (fig. 3), the apparent and total flux increased linearly.

Discussion

In recent years much evidence has been accumulated regarding the possible role of HDL in clearing cellular cholesterol from peripheral tissues in order to transport it to the liver (18). A key step in this process seems to be the esterification of free cholesterol catalyzed by LCAT (7). The final destiny of this CE is the liver, which can dispose of excess cholesterol via biliary secretion. However, plasma CE can be transferred among lipoproteins (16, 20), and therefore, the amount of them that will reach the liver or that stay in plasma LDL will depend on their rate of transfer. Our results confirm that LDP stimulates the exchange of CE between LDL and HDL (fig. 1) and that this exchange is inhibited linearly by the amount of HDL present in the assay (fig. 2). Flux calculations of CE between both lipoproteins show the same thing (Table II).

BARTER and JONES (3) have suggested that the exchange or transfer factor not only has the capacity of exchanging CE between two diferent lipoprotein fractions but also between two molecules within the same lipoprotein class and that HDL in high concentrations binds this protein to such an extent that exchange between HDL and LDL is decreased. However, our data support the argument that the limiting factor is not the absolute concentration of HDL, because when LDL concentration was increased simultaneously, the transfer of CE augmented linearly (fig. 3). The fact that HDL inhibits CE transfer coincides with epidemiological data which indicate that high HDL concentration in circulating blood has a protective effect against atherosclerosis (14). Nevertheless, more important than the concentration of HDL by itself seems to be the HDL: LDL ratio.

Resumen

Se estudia *in vitro* el intercambio de ésteres de colesterol entre el HDL y otras lipoproteínas plasmáticas, estimulado por plasma libre de lipoproteínas. Los resultados indican que el intercambio es inhibido con el aumento de concentración de HDL presente en el ensayo. Sin embargo, el factor limitante no es la concentración absoluta de HDL, dado que cuando se aumenta simultáneamente la cantidad de LDL, el intercambio aumenta proporcionalmente al *pool* total de ésteres de colesterol. Se discuten las implicaciones de estos datos sobre el metabolismo global del colesterol plasmático.

References

1. ABELL, L. L., LEVY, B. B. and KENDALL, F. E.: *J. Biol. Chem.*, **195**, 357-366, 1952.
2. AKANUMA, Y. and GLOMEST, J.: *J. Lipid Res.*, **9**, 620-626, 1968.
3. BARTER, P. J. and JONES, M. E.: *J. Lipid Res.*, **21**, 238-249, 1980.
4. BARTER, P. J. and LALLY, J. I.: *Metabolism*, **28**, 230-236, 1979.
5. BURSTEIN, M., SCHOLNICK, H. R. and MORFIN, R.: *J. Lipid Res.*, **11**, 583-595, 1970.
6. GLOMSET, J. A.: *J. Lipid Res.*, **9**, 155-167, 1968.
7. GLOMSET, J. A.: *Amer. J. Clin. Nutr.*, **23**, 1129-1136, 1970.
8. GOODMAN, Z. D. and LEQUIRE, V. S.: *Biochim. Biophys. Acta*, **398**, 325-336, 1975.
9. GORNALL, A. G., BARDAWILL, C. S. and DAVID, M. M.: *J. Biol. Chem.*, **177**, 751-766, 1949.
10. HAGEMAN, J. S. and GOULS, R. G.: *Proc. Soc. Exp. Biol. Med.*, **78**, 329-332, 1951.
11. HATCH, F. and LEES, R.: *Adv. Lipid Res.*, **6**, 1-68, 1968.
12. HAVEL, J. J., EDER, H. A. and BRADON, S. H.: *J. Clin. Invest.*, **34**, 1345-1353, 1971.
13. MAHLEY, R. W. and INNERARITY, T. L.: *Biochim. Biophys. Acta*, **737**, 197-222, 1983.
14. MILLER, G. J.: *Ann. Rev. Med.*, **31**, 97-108, 1980.
15. NORUM, K. R. and GJONE, E.: *Biochim. Biophys. Acta*, **144**, 698-700, 1967.
16. PATTNAIK, N. M., MONTES, A., HUGHES, L. B. and ZILVERSMIT, D. B.: *Biochim. Biophys. Acta*, **530**, 428-438, 1978.
17. STEIN, O., VANDERHOEK, J., FRIEDMAN, G. and STEIN, Y.: *Biochim. Biophys. Acta*, **450**, 367-378, 1976.
18. TALL, A. R. and SMALL, D. M.: *Adv. Lipid Res.*, **17**, 1-51, 1980.
19. ZAK, B., MOS, N., BOYLE, A. J. and ZLATKIS, A.: *Anal. Chem.*, **26**, 776-777, 1954.
20. ZILVERSMIT, D. B., HUGHES, L. B. and BALMER, J.: *Biochim. Biophys. Acta*, **409**, 393-398, 1975.