Vol. 94, No. 1, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1980 Pages 101-108

DEGRADATION OF HIGH DENSITY LIPOPROTEIN BY HEPARIN-RELEASABLE LIVER LIPASE

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Received March 17,1980

SUMMARY In vivo inhibition of heparin-releasable liver lipase (liver lipase) induces a change in the chemical composition of a subfraction of high density lipoprotein (HDL_2; density range 1.05-1.13 g/ml). HDL_2 becomes rich in phospholipids and relatively poor in protein and cholesterolesters. Incubation of this phospholipid-rich HDL_2 or control HDL_2 with purified liver lipase at pH 7.4 gives a marked hydrolysis of phosphatidylcholine and phosphatidylethanolamine, but not of sphingomyelin and cholesterolesters. The bulk of lysophosphatidylcholine formed during incubation is recovered in the fraction of density > 1.21 g/ml. Phospholipid-rich HDL_2 is not converted to HDL_3 (density range 1.13-1.21 g/ml), but to a cholesterol(ester)-rich HDL_2. This is probably due to the absence of an acceptor for cholesterol(esters) in this in vitro system. In vivo, however, liver lipase could function in the conversion of HDL_2 into HDL_3, because the cholesterol(esters) can be transferred to other lipoproteins and/or tissues.

Plasma triacylglycerol is hydrolyzed by lipoprotein lipase (EC 3.1.1.3), located at the capillary endothelial surface of various tissues. This exoenzyme can be released by intravenous injection of heparin or similar polyanions[1]. In addition to lipoprotein lipase, post-heparin plasma contains a distinct lipase of hepatic origin[2,3]. This heparin-releasable lipase from liver (liver lipase) is able to hydrolyze (tri)acylglycerols, phospholipids and long-chain acyl-CoA in vitro[2-6] and also catalyzes transacylation reactions[7,8].

The activity of liver lipase does not correlate with the plasma level of triacylglycerol[9], but (negatively) with plasma HDL-cholesterol(esters) in healthy subjects[10]. We and others showed that in vivo inhibition of liver lipase in rats, by intravenous injection of a specific antibody, has profound effects on the levels of serum lipoproteins[11,12], especially HDL. The antibody treatment caused a change in the distribution of HDL subfractions e.g. an increase in HDL_2-phospholipids and-cholesterol and a decrease in HDL_3-lipids[12]. In the light of these experiments it became necessary to know if and how these high levels of HDL_2 are degraded again by purified liver lipase in vitro.

Abbreviations: HDL_2, high density lipoprotein (density range 1.05-1.13 g/ml); HDL_3, high density lipoprotein (density range 1.13-1.21 g/ml); LCAT, lecithin cholesterol acyltransferase.

0006-291X/80/090101-08$01.00/0

MATERIALS AND METHODS

Isolation of (phospholipid-rich) HDL_2. Male Wistar rats were used, after an overnight fast (body weight 200-250 g). HDL_2 was isolated as described before[12]. Phospholipid-rich HDL_2 was obtained from rats injected with a specific antibody against (heparin-releasable) liver lipase[12].

Incubation of HDL_2 with purified (heparin-releasable) liver lipase. The liver lipase was purified from rat post-heparin plasma by affinity chromatography on Sepharose-4B, containing covalently-bound heparin, as described before[13,14]. The enzyme was eluted with 0.8 M NaCl, containing 10 mM phosphate buffer pH 7.0 and 5% ($^{V}/v$) glycerol. The activity was 2.34 U/ml, if measured at 30°C with palmitoyl-CoA as the substrate[13]. HDL_2 (0.9 or 1.6 mg of phospholipid) was incubated for 1 or 2 h at 37°C and pH 7.4 in the presence or absence of 0.5 U of purified liver lipase. The incubation medium contained 20 mM Tris-HCl, 1 mM $CaCl_2$, 0.6 mM P_i, 125 mM NaCl, 2.5% ($^{W}/v$) bovine serum albumin (Sigma, fraction V, fatty acid-free) and 0.3% glycerol. Phospholipid analyses were performed on trichloroacetic acid precipitates of the incubation medium. The medium was also brought to a density of 1.30 g/ml with solid KBr and fractionated exactly as during the isolation of lipoproteins from serum[12], resulting in fractions of density <1.02 g/ml, 1.02-1.05 g/ml, 1.05-1.13 g/ml, 1.13-1.21 g/ml and >1.21 g/ml.

Chemical analyses of lipoprotein (sub)fractions. Protein was determined according to Lowry et al.[15], using bovine serum albumin as a standard. Cholesterol plus cholesterolesters and phospholipids were measured as described in refs. 16 and 17, respectively. For the assay of unesterified cholesterol the cholesterolester hydrolase was omitted from the assay medium. Cholesterolesters were calculated and expressed as the difference between total and unesterified cholesterol. The different phospholipid species were assayed after separation by thin layer chromatography using chloroform/aceton/methanol/acetic acid/water (10/4/2/2/1; $^{V}/v$). The various phospholipids were eluted from the silica gel and measured as phospholipid phosphorus. Radioactive standards were included for recovery calculations (c.f. ref. 18).

RESULTS

Table I gives the chemical composition of control HDL_2 and of phospholipid-rich HDL_2 from animals pretreated in vivo with a specific antibody against liver lipase. The ratios of phospholipid/protein and phospholipid/cholesterolester are significantly increased after antibody treatment, while the ratio of phospholipid/cholesterol remained unchanged. The ratio of cholesterolester/cholesterol showed a decrease, which, however, only approached statistical significance ($P<0.08$). As found earlier[12], there was no change in the activity of the serum enzyme LCAT by the antibody treatment.

Fig. 1 shows the formation of lysophosphatidylcholine from phosphatidylcholine during incubation of HDL_2 with purified liver lipase. The reaction is not linear with time during the 2 h of incubation. This is (partly) due to inactivation of the enzyme, as the palmitoyl-CoA hydrolase activity also decreased (see legend to Fig. 1). Sphingomyelin is not catabolized to a significant extent. Phospholipid-rich HDL_2 contains a small amount of phosphatidylethanolamine, in contrast to control HDL_2, which is degraded completely during the first hour of incubation. Apart from this, the fractional degradation rates of control HDL_2 and phospholipid-rich HDL_2 are about the same.

TABLE I COMPOSITION OF HDL_2 IN CONTROL RATS AND RATS PRETREATED WITH ANTIBODY AGAINST LIVER LIPASE

The chemical composition is given as weight % ± SEM (n=5).

	Control rats	Antibody-treated rats
Protein	38.2±2.0	33.5±0.5*
Phospholipid	34.4±1.7	41.8±0.5**
Cholesterolester	22.1±1.0	17.9±0.6**
Cholesterol	5.2±0.5	5.9±0.3
Triacylglycerol	1.2±0.6	0.9±0.0
Ratio $\frac{\text{phospholipid}}{\text{protein}}$	0.90±0.01	1.25±0.08***
Ratio $\frac{\text{phospholipid}}{\text{cholesterolester}}$	1.56±0.09	2.34±0.07***
Ratio $\frac{\text{phospholipid}}{\text{cholesterol}}$	6.62±0.59	7.08±0.32
Ratio $\frac{\text{cholesterolester}}{\text{cholesterol}}$	4.25±0.51	3.03±0.21
Ratio $\frac{\text{surface}}{\text{core}}$	3.29±0.21	4.32±0.15**

*** Significant difference from control rats $P<0.001$, using Student's t-test
** $P<0.005$
* $P<0.02$

Fig. 2 shows that HDL_2 protein and cholesterol(ester) are not transferred to other density ranges to any significant extent by incubation with liver lipase. If reisolated after incubation, these constituents are almost complete-

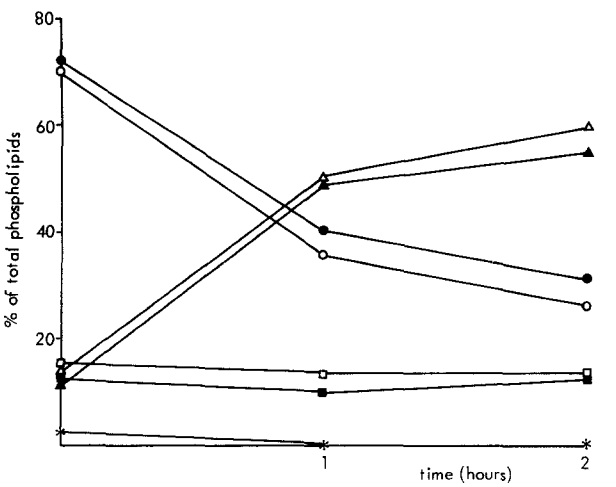

Fig. 1. Degradation of HDL_2-phospholipids by purified heparin-releasable liver lipase. The relative amounts of phospholipids in the total incubation medium are plotted against time. Phosphatidylcholine (●-o); phosphatidylethanolamine in phospholipid-rich HDL_2 (✱); sphingomyelin (■-□); lysophosphatidylcholine (▲-△). Control HDL_2 (o-△-□); phospholipid-rich HDL_2 from antibody-treated rats (●-▲-■). The liver lipase activity, measured with palmitoyl-CoA as substrate after 1 or 2 h of incubation, was 82% and 34% of the initial values, respectively (average of 2-3 separate experiments). For further details see Materials and Methods.

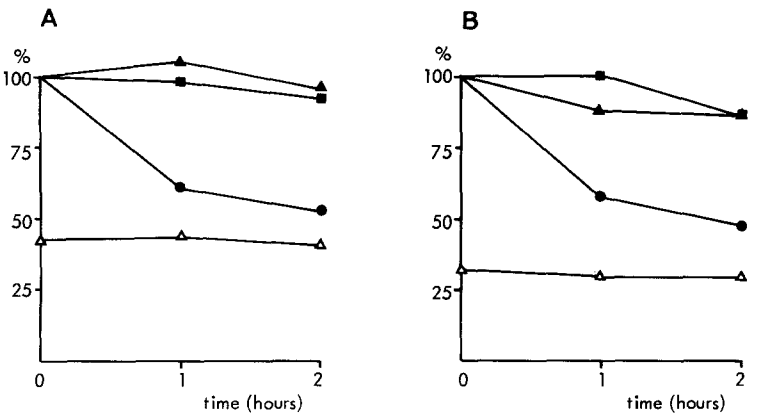

Fig. 2. Effect of incubation of HDL_2 with purified liver lipase on the concentration of total phospholipids (●), cholesterol plus cholesterolesters (▲) and protein (■) in the HDL_2 density fraction (1.05-1.13 g/ml) expressed as percentage of the initial concentration. A: phospholipid-rich HDL_2, isolated from antibody-treated rats. B: control HDL_2. The open triangles (△) give the % of total cholesterol which is in the unesterified form at the incubation times indicated. For further details see Materials and Methods and the text.

ly recovered in the HDL_2 density range. The relative amounts of cholesterol and cholesterolester are constant during incubation, indicating that liver lipase does not act as a cholesterolesterase, under the conditions used. Phosphatidylcholine is progressively lost from HDL_2 during the incubation.

The chemical composition of HDL_2 changes by incubation with liver lipase, as can be expected from the data of Figs. 1 and 2. This change is illustrated in Fig. 3. The diagram clearly shows the difference between the composition of control HDL_2 and phospholipid-rich HDL_2 before incubation. Triacylglycerol

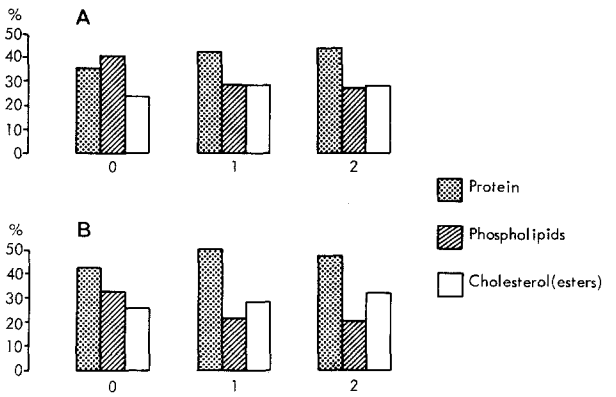

Fig. 3. Chemical composition of HDL_2 (reisolated from the density range of 1.05-1.13 g/ml) before and after 1 or 2 h of incubation with purified liver lipase. The values are given as weight %. A: phospholipid-rich HDL_2 isolated from antibody-treated rats. B: control HDL_2. The HDL_2 composition was not changed by incubation at 37°C in the absence of liver lipase. For details see Materials and Methods and the text.

TABLE II DENSITY DISTRIBUTION OF PHOSPHOLIPIDS AFTER INCUBATION OF HDL_2 IN THE PRESENCE OR ABSENCE OF PURIFIED LIVER LIPASE

Incubation conditions	Density range (g/ml)	Percentage of total phospholipid-phosphorus added to the incubation*				
		Total	PE	PC	Lyso PC	SM
1 h at 37°C without liver lipase	1.02-1.05	8.7 (7.8)	0.2 (-)	7.1 (6.3)	- (-)	1.4 (1.5)
	1.05-1.13	73.2 (69.9)	1.8 (0)	59.7 (56.0)	0 (0)	11.7 (13.9)
	1.13-1.21	3.6 (0)	- (-)	1.6 (0)	1.2 (0)	0.8 (0)
	>1.21	9.7 (12.6)	- (-)	2.3 (3.0)	7.4 (9.6)	0 (0)
1 h at 37°C in the presence of liver lipase	1.02-1.05	2.8 (3.1)	- (-)	2.2 (2.2)	- (-)	0.6 (0.9)
	1.05-1.13	44.5 (40.8)	0 (0)	32.5 (28.9)	2.9 (0)	9.1 (11.9)
	1.13-1.21	7.1 (6.0)	- (-)	2.7 (2.6)	3.0 (1.3)	1.4 (2.0)
	>1.21	43.8 (39.8)	- (-)	7.6 (3.7)	36.2 (36.1)	0 (0)
2 h at 37°C in the presence of liver lipase	1.02-1.05	2.1 (1.6)	- (-)	1.6 (1.1)	- (-)	0.5 (0.5)
	1.05-1.13	38.3 (34.0)	- (-)	27.4 (21.4)	3.1 (2.7)	7.8 (9.9)
	1.13-1.21	9.9 (3.7)	- (-)	6.0 (1.6)	2.5 (1.2)	1.3 (0.8)
	>1.21	44.2 (38.4)	- (-)	1.5 (1.0)	40.3 (36.3)	2.5 (1.1)

* The values given are obtained with phospholipid-rich HDL_2 from antibody-treated rats or with control HDL_2 (in parentheses). - indicates that the fraction was not assayed because the level was too low for detection. PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin.

contributes only very little to rat HDL_2 (see Table I) and was therefore not included in the diagram. Both control HDL_2 and phospholipid-rich HDL_2 are converted into HDL_2, which is enriched in cholesterol(esters).

Table II gives the distribution of phospholipids in the various density fractions. The lysophosphatidylcholine formed during incubation is recovered mainly at densities >1.21 g/ml, probably bound to albumin. Sphingomyelin, which is not degraded (see Fig. 1), is transferred to some extent from densities <1.13 g/ml to densities >1.13 g/ml and even >1.21 g/ml. In addition to hydrolysis of phosphatidylcholine, a small part is transferred unhydrolyzed to higher densities, quite analogous to sphingomyelin. Some phosphatidylcholine and lysophosphatidylcholine are present in the >1.21 g/ml density fraction, even after incubation of HDL_2 in the absence of liver lipase. A small amount of lysophosphatidylcholine is already present in HDL_2 before incubation. It cannot be decided at the moment whether this lysophosphatidylcholine is an intrinsic component of rat HDL_2 or is formed during isolation (e.g. by LCAT).

DISCUSSION

From the data shown in Table I and those published earlier[12], it is concluded that the HDL_2 isolated from antibody-treated rats is enriched in phospholipid and relatively poor in protein and cholesterolester. HDL_2 probably is a spherical particle like HDL_3[19], consisting of an hydrophobic core of cholesterolester and triacylglycerol which is surrounded by a surface of phospholipid, cholesterol and protein. The antibody treatment results in a significant increase of the ratio of surface/core material (see Table I). This does not necessarily have consequences for the shape of the HDL_2 particle as Jonas found that HDL can accomodate extra phospholipid and/or cholesterol[20].

Earlier we concluded that the primary effect of in vivo inhibition of liver lipase was an increase in HDL_2 phospholipids[12]. As all lipids in the HDL_3 fraction dropped in the antibody-treated animals, we suggested that the heparin-releasable liver lipase could play a role in the conversion of HDL_2 into HDL_3 in vivo. The present experiments support this hypothesis, as they show that purified liver lipase is capable of HDL_2-phospholipid degradation in vitro. This is rather specific for liver lipase as lipoprotein lipase from bovine milk does not hydrolyze HDL phospholipids[21]. The phospholipid-rich HDL_2 is not converted into HDL_3 in the present in vitro experiments, but into HDL_2, which is enriched in cholesterol(esters). The same is observed with control HDL_2 (see Fig. 3). This is probably due to the absence of a suitable acceptor for cholesterol(ester) in vitro. If cholesterol(ester)-rich HDL_2 circulates in vivo, it can be expected, however, to donate its excess cholesterol(ester) to other lipoproteins (a process possibly involving a cholesterolester transfer protein [22]) and/or tissues. This could result in the formation of HDL_3 (see Fig. 4).

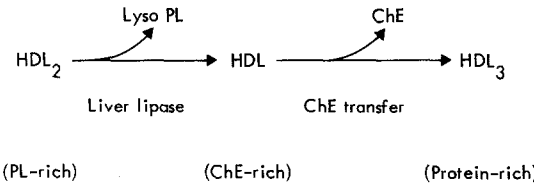

Fig. 4. Hypothesis for the in vivo conversion of HDL_2 into HDL_3, involving liver lipase activity and cholesterol(ester) transfer. For further explanation see the text. PL, phospholipids; ChE, cholesterol(esters).

Our data, together with the negative correlation between HDL-cholesterol-(esters) and liver lipase activity found in normal human subjects[10], certainly suggest a function of heparin-releasable liver lipase in the removal from serum of HDL-cholesterol(esters) as well as HDL-phospholipids. The liver lipase is located at the external surface of sinusoidal liver cells[14,23]. These cells are also active in the uptake of lipoprotein cholesterolester from the circulation[24]. A fraction of the serum HDL-cholesterol(esters) could be channelled to the liver by the phospholipase action of liver lipase. In this way liver lipase could function, in combination with LCAT, in the centripetal transport of cholesterol from the perifery to the liver[25]. A high liver lipase activity, as present in adult males compared to females[26], could also induce, by active hydrolysis of HDL_2-phospholipids, the transfer of cholesterol(esters) to other lipoproteins (e.g. low density lipoproteins) or extrahepatic endothelial cells. The relatively high activity in males also explains the low levels of HDL_2 in males compared to females[27].

ACKNOWLEDGEMENTS

Prof.Dr. W.C. Hülsmann and Miss A.C. Hanson are gratefully thanked for critically reading and preparing the manuscript, respectively. The stimulating discussions with Dr. P.H.E. Groot and the expert technical assistance by Miss C. Kalkman are also gratefully acknowledged.

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