

## SELECTIVE DEGRADATION OF THE HIGH DENSITY LIPOPROTEIN-2 SUBFRACTION BY HEPARIN-RELEASABLE LIVER LIPASE

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### 1. Introduction

The lipase released from the liver after intravenous injection of heparin (liver lipase, EC 3.1.1.3) has a broad substrate specificity [1–3]. Hydrolysis of ester bonds in triacylglycerol, diacylglycerols, 1 or 2 monoacylglycerols, palmitoyl-coenzyme A and phosphatidylcholine (the 1 acyl ester bond) has been demonstrated, using artificial lipid emulsions. Although a role in serum lipoprotein metabolism has generally been considered, the physiological function of this enzyme has not been well established. Evidence has been presented, based on *in vivo* inhibition of liver lipase by intravenous injection of a specific antiserum in rats, that liver lipase is involved in HDL- and possibly LDL-phospholipid catabolism [4–6]. Based on these experiments a role of liver lipase in the conversion of HDL-2 into HDL-3 was proposed. This hypothesis was developed further by *in vitro* incubations of liver lipase with rat HDL-2 [7] and by *in vivo* turnover studies of HDL-phospholipids and cholesterol esters [8]. Here, the hypothesis was further investigated. As HDL-2 and HDL-3 are poorly defined in rat, we turned to human serum and measured the phospholipase A activity of purified human heparin-releasable liver lipase with isolated HDL-2 and HDL-3. It was found that the rate of phosphatidylcholine hydrolysis with HDL-2 as substrate exceeds the rate with HDL-3 by a factor of ~10. These data provide further evidence for a role of liver lipase in the degradation of HDL-2.

**Abbreviations:** HDL, high density lipoprotein; LDL, low density lipoprotein; PLEP, phospholipid exchange protein; VLDL, very low density lipoprotein

### 2. Materials and methods

#### 2.1. The purification of human liver lipase

Post-heparin blood was obtained from fasted volunteers, 6 min after the intravenous injection of heparin (50 U/kg body wt) and plasma was obtained by low speed centrifugation. Liver lipase was purified from the plasma by affinity chromatography on Sepharose-4B containing covalently bound heparin [9]. The enzyme was eluted with a linear NaCl gradient (0.2–2 M) in 5 mM sodium barbital (pH 7.4) containing 20% (v/v) glycerol. Liver lipase (eluted at ~0.8 M NaCl) was completely separated from lipoprotein lipase (eluted at ~1.3 M NaCl) and its specific activity increased ~250-fold. An additional 5-fold purification was accomplished on a second heparin–Sepharose column operated exactly as in [10]. Top fractions, eluted at ~0.8 M NaCl concentration, hydrolyzed a triolein substrate with spec. act. 4.3  $\mu\text{mol}$  fatty acid released  $\cdot \text{min}^{-1} \cdot \text{mg protein}^{-1}$ , using the assay conditions in [3], including 1 M NaCl (table 1). The enzyme was stored at  $-20^{\circ}\text{C}$  in 0.8 M NaCl, 5 mM sodium barbital buffer (pH 7.4), 0.2% bovine serum albumin and 20% (v/v) glycerol. Before use the enzyme was concentrated and dialyzed against 0.9% NaCl, 5 mM sodium barbital buffer (pH 7.4) using a hollow fiber.

#### 2.2. Preparation of human lipoproteins labeled *in vitro* in their phosphatidylcholine moiety

Fresh human sera of fasted, healthy, female donors were labeled in their phosphatidylcholine moiety by exchange with phosphatidyl[*methy*l- $^3\text{H}$ ]choline present in purified rat liver microsomes, using the phosphatidylcholine exchange protein from bovine liver (PC-PLEP), essentially as has been described [11]. Phosphatidyl[ $^3\text{H}$ ]choline labeled rat liver microsomes

Table 1  
The purification of human liver lipase

Fraction	Vol. (ml)	Protein (mg)	Lipase activity (U)	Spec. act. (U/mg protein)	Recovery (%)
Post-heparin plasma	90	5400	18	0.0033	100
Peak activity 1st heparin-Sephadex column	83	6.6	5.58	0.84	31
Peak activity 2nd heparin-Sephadex column	27	0.39	1.7	4.3	9.4

were prepared by incubation of microsomes with [*methyl*-<sup>3</sup>H]choline in the presence of 0.16 mM CaCl<sub>2</sub> [12]. Microsomes (2.2 μmol phospholipid containing 8.8 × 10<sup>6</sup> dpm), human serum (6 ml containing ~20 μmol lipoprotein phospholipid) and pure PC-PLEP [13] (0.045 mg; a generous gift from Dr K. W. A. Wirtz) were mixed and incubated for 1 h at 37°C. Next the microsomes were spun down (1 h at 47 000 rev./min in a Beckman 50 rotor at 4°C). About 90% of the radioactivity was associated with serum lipoproteins, mainly (>95%) with phosphatidylcholine. Net transfer of phospholipid did not occur during our labeling procedure as the phospholipid phosphorus concentrations in serum and microsomes were not affected by the incubation.

### 2.3. Isolation of HDL-2 and HDL-3

HDL-2 and HDL-3 were isolated from prelabeled sera by density gradient ultracentrifugation, essentially as in [14]. Serum lipoprotein separation was accomplished by centrifugation for 45 h at 40 000 rev./min at 15°C in the Beckman SW 40 rotor. After centrifugation two HDL bands were clearly visible in the lower part of the tube and were harvested by tube slicing at predetermined positions. The density profile after centrifugation was determined in control gradients using a precision density meter (Anton Paar, Graz, model DMA 40) and used to determine the cut-off densities of the HDL-2 and HDL-3 fractions (HDL-2, 1.07 < *d* < 1.11 g/ml; and HDL-3, 1.11 < *d* < 1.175 g/ml). Lipoprotein fractions were dialyzed against 0.9% NaCl, 1 mM EDTA, 10 mM Tris-HCl (pH 7.4), 0.1% NaN<sub>3</sub> and stored at 4°C under nitrogen.

### 2.4. Incubation of HDL fractions with purified liver lipase

HDL (0.25 mM phospholipid) was incubated with

or without liver lipase (~10 mU if measured with triolein as substrate) in a medium containing 150 mM NaCl, 40 mM Tris-HCl (pH 7.4), 0.5 mM CaCl<sub>2</sub> and 1% defatted bovine serum albumin for 30 or 60 min in a final volume of 0.1 ml at 37°C. The incubation was terminated by a chloroform-methanol extraction as in [15]. The radioactivity in the chloroform phase associated with lysophosphatidylcholine was determined after the separation of phospholipids by thin layer chromatography on Silica gel using chloroform-methanol-acetic acid-water (100:50:18:8, by vol.).

### 2.5. Other methods

Triacylglycerol lipase activity of liver lipase was determined with a triolein emulsion stabilized by gum acacia as in [3], except that 1 M NaCl was used. The enzyme activity is expressed in μmol fatty acid released/min incubation at 30°C.

Phospholipid phosphorus, protein, cholesterol and cholesterol esters were determined as in [7].

## 3. Results and discussion

Human HDL fractions were labeled *in vitro* in their phosphatidylcholine moiety using a specific exchange protein isolated from bovine liver which facilitates exchange of phosphatidylcholine between membranes and lipoproteins [11]. The chemical composition of phosphatidyl[<sup>3</sup>H]choline-labeled HDL-2 and HDL-3 is shown in table 2. HDL-2 is enriched in all lipid components and poor in protein in comparison with HDL-3, as has been shown before for HDL-2 and HDL-3 separated by zonal ultracentrifugation [16,17]. The concentration of HDL-2 is ~1/2 that of HDL-3 in the sera of our (female) blood donors see legend table 2; a slightly lower ratio was described in [16] in a normal female population.

Table 2  
Chemical composition (weight %  $\pm$  SD,  $n = 4$ ) of human HDL-2 and HDL-3 labeled in the phosphatidylcholine moiety

	Chemical composition			
	Protein	Phospholipid	Free cholesterol	Cholesterol ester
HDL-2	38.4 $\pm$ 2.89	34.3 $\pm$ 2.24	4.9 $\pm$ 0.90	22.3 $\pm$ 4.10
HDL-3	52.8 $\pm$ 1.07	26.6 $\pm$ 2.59	2.5 $\pm$ 0.12	18.1 $\pm$ 2.86

For details see section 2. The specific radioactivity of phosphatidylcholine in HDL-2 and HDL-3 was 397  $\pm$  32 and 441  $\pm$  46 dpm/nmol phospholipid phosphorus, respectively. The serum concentrations of HDL-2 and HDL-3, calculated from the chemical composition, were 1.15  $\pm$  0.34 and 2.17  $\pm$  0.26 mg/ml serum, respectively. Molecular masses of phospholipid and cholesterol esters in HDL were assumed to be 775 and 644, respectively, HDL triglycerides were not measured

Isolated HDL-2 and HDL-3 fractions were incubated with liver lipase and the generation of radio-labeled lysophosphatidylcholine was determined by thin-layer chromatography. The results are shown in table 3. The rate of HDL-2 phosphatidylcholine hydrolysis exceeded that of HDL-3 by a factor of 5 or more in all individual experiments. Under the conditions, shown in table 3, a linear relationship between incubation time and lysophosphatidylcholine formation was found (not shown). To determine the kinetic parameters for HDL-2 phosphatidylcholine hydrolysis by human liver lipase, a substrate saturation study was undertaken. The results are shown in fig.1. The app.  $K_m$  for HDL-2 phosphatidylcholine hydrolysis (0.1 mM) is lower than the serum concentration (0.1–0.40 mM HDL-2 phospholipid phosphorus). Our

assay system did not allow an accurate measurement of the  $K_m$  for HDL-3.

Human HDL-2 and HDL-3 are interrelated. A HDL-2 like particle can be formed from HDL-3 in vitro during VLDL catabolism by lipoprotein lipase [18] and an increased lipolysis in man in vivo has also been connected with an increased HDL-2 concentration [19]. HDL-2 particles are larger and contain 2–3-times more phospholipid, cholesterol and cholesterol ester than HDL-3 particles [20]. The conversion of HDL-3 into HDL-2 could be the result of uptake of surface lipids and apolipoproteins (C and A-1) from VLDL and chylomicrons during lipolysis, followed by cho-

Table 3  
Hydrolysis of phosphatidyl[ $^3$ H]choline on isolated HDL-2 and HDL-3 by human liver lipase

	Lysophosphatidylcholine formed (nmol/h) mean $\pm$ SD ( $n = 4$ )
HDL-2	1.90 $\pm$ 0.14
HDL-3	0.16 $\pm$ 0.19

HDL-2 or HDL-3 were incubated with or without human liver lipase (8–16.6 mU as measured with a triolein substrate on the day of the experiment) and the liver lipase-dependent lysophosphatidylcholine formation was determined as in section 2. For comparison the data are normalized as if 16.6 mU liver lipase (with triolein as substrate) had been added in all experiments. HDL phospholipid phosphorus was 0.25 mM; incubation was for 60 min

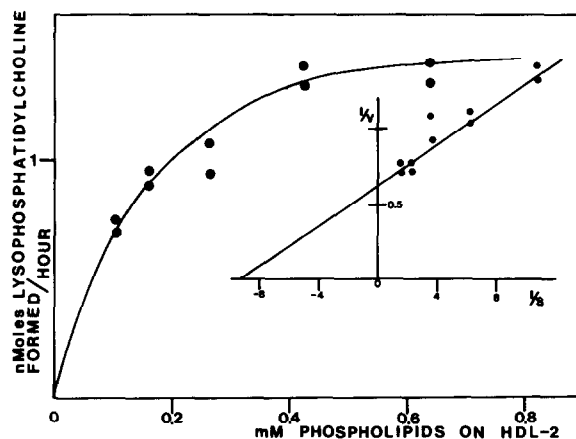


Fig.1. Substrate saturation of HDL-2 phosphatidyl[ $^3$ H]choline hydrolysis by human liver lipase. Insert: Lineweaver-Burk plot ( $1/V$  vs  $1/S$ ) of the data. The  $K_m$  calculated from the data was 0.1 mM HDL-2 phospholipid phosphorus; incubation time 60 min.

lesterol esterification by lecithin:cholesterol acyltransferase. A scheme has been proposed for the reverse process, e.g., the conversion of HDL-2 into HDL-3 [5–8]. In that scheme liver lipase hydrolyzes HDL-2 phosphatidylcholine. As the surface of HDL-2 shrinks by such process, its cholesterol and cholesterol esters may be transferred, possibly to organs equipped with a liver lipase (in addition to the liver also present in the adrenals and ovaries [21,22]) or to other plasma lipoproteins. These data support that hypothesis, as HDL-2 phosphatidylcholine is a superior substrate for liver lipase compared to HDL-3. The origin of this preference is not yet understood. It may be connected with differences in the composition of the apolipoprotein moiety of HDL-2 (a higher content of apo C peptides and apo E, a higher apo A-I/apo A-II ratio) relative to HDL-3. However in preliminary studies we were unable to demonstrate effects of addition of apo E and apo C-I to the incubation medium on HDL-3 phosphatidylcholine hydrolysis. Alternatively the physical state of HDL (i.e., radius of curvature or surface pressure) could determine the rate of phospholipid hydrolysis but obviously more experiments are needed to clarify this point.

HDL-2 may be a subfraction of HDL which links several pathways of lipoprotein metabolism. However, HDL-3 is often taken to be representative for total plasma HDL. The big difference in properties between the HDL subfractions, as described here, stresses the importance of the use of HDL-2 in addition to HDL-3 in metabolic and cell interaction studies.

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