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Phospholipid Composition of High-Density Lipoproteins Reflects Lipolysis of Triglyceride-Rich Lipoproteins during Hyperlipidemia

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We studied phospholipid composition of high-density lipoproteins in patients with normo- and hypertriglyceridemia treated with various hypolipidemic preparations (simvastatin and fenofibrate). Both preparations changed phospholipid composition of high-density lipoproteins and improved their functional activity. The differences in the phospholipid composition of high-density lipoproteins were probably related to lipolysis of triglyceride-rich lipoproteins catalyzed by lipoprotein lipase and, in particular, hepatic lipase.

Key Words: *lipoproteins; phospholipids; lipoprotein lipase; hepatic lipase*

High-density lipoproteins (HDL) accept cholesterol (CH) from cell membranes in peripheral tissues and from very-low- (VLDL), intermediate-, and low-density lipoproteins (LDL) [15]. Therefore, studies of HDL components determining this property are of considerable interest.

It was demonstrated that the negative correlation between plasma contents of HDL CH and triglycerides (TG) is determined by two key lipolytic enzymes, peripheral lipoprotein lipase and hepatic lipase modulating the composition and transformations of circulating lipoproteins [8]. Lipoprotein lipase cleaves TG in VLDL and chylomicrons. Surface fragments of these particles containing phospholipids (PL) and free (non-esterified) CH are accepted by HDL, while VLDL and chylomicrons are converted into intermediate-density lipoproteins. Hepatic lipase catalyzes the conversion of intermediate-density lipoproteins into LDL [13] and transformation of large HDL particles into small particles [5,6,9]. In hypertriglyceridemia, large HDL particles with TG-enriched core are good substrate for hepatic lipase. Hepatic lipase hydrolyzes TG and PL of HDL; the relative content of HDL CH increases, which reduces the efficiency of reverse CH transport to the liver [4,9,12]. The relationship between lipoprotein and hepatic lipases plays an important role in plasma TG transport and modulates the concentration and composition of atherogenic VLDL and LDL and antiatherogenic HDL [13].

We studied the dependence of PL composition of HDL on the content of TG to evaluate the involvement of lipoprotein and hepatic lipases into HDL modifications. Changes in plasma lipoproteins were induced by two hypolipidemic drugs: simvastatin (Zokor, MSD), inhibitor of key enzymes of CH synthesis, and micronized fenofibrate (Lipantil 200 M, Laboratories Fournier), selective activator of lipoprotein lipase.

MATERIALS AND METHODS

We examined 53 male patients (40-59 years) with coronary heart disease and hyperlipidemia (LDL CH≥160 mg/dl) persisting after 2-week hypolipidemic diet.

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Some patients (n=26) were treated with simvastatin in a daily dose of 10-20 mg, while others (n=27) received fenofibrate in a daily dose of 200 mg for 8 weeks.

Before and after the therapy, venous blood was taken after 8-10-h fast. Plasma CH and TG contents were measured on a Tsentrifikhem 600 autoanalyzer using enzyme kits. HDL CH concentration was measured in the supernatant after precipitation of plasma VLDL and LDL with sodium phosphotungstate and magnesium chloride [3] (similarly to measurements of total CH content). The concentration and composition of HDL PL were estimated after precipitation of plasma LDL. PL were extracted with a 2:1 chloroform-methanol mixture [7]. HDL PL content was measured after mineralization and reaction with ammonium molybdate and ascorbic acid [14]. PL were separated by thin layer chromatography on glass plates coated with silica gel in a chloroform-methanol-aqua ammonia-water mixture (17:7:1:0.5 v/v) [1]. Plates were developed in iodine vapors, the zones corresponding to individual PL were scraped, and after mineralization phosphorus concentration was estimated in the reaction with hydrochloric hydrazine and sodium molybdate [16]. The data were expressed in percents of total PL content.

The results were analyzed by Student's t test and pairwise Wilcoxon test. The differences were significant at $p<0.05$.

RESULTS

There were 2 groups of patients with normal (below 180 mg/dl) and high plasma TG contents (equal or above 180 mg/dl). The mean content of total CH did not differ in these groups (Table 1).

Patients with normal plasma TG concentration had higher contents of HDL CH and HDL PL. In these patients HDL were enriched with phosphatidylcholine (PC) and phosphatidylethanolamine (PE), while the content of lysophosphatidylcholine in HDL was lower than in patients with high TG concentration (Table 1).

Simvastatin decreased plasma CH content in patients with normal and high TG concentrations by 26.6 and 25.8%, respectively. Plasma TG content decreased only in patients with high TG concentration (by 18.8%). The content of HDL CH insignificantly increased in patients of both groups. HDL PL content increased in patients with normal and high TG concentrations by 10.9 and 6.4%, respectively (Table 2).

Fenofibrate decreased plasma CH content in patients with normo- and hypertriglyceridemia by 22.4 and 16.5%, respectively (Table 2). TG content decreased in patients with normal and high TG concentrations by 30 and 49%. The increase in HDL CH content was more pronounced in patients with hypertriglyceridemia (16.3%, from 43.0±2.8 to 50.0±2.1 mg/dl, $p<0.01$) compared to patients with normal TG concentration (4.1%, from 49.0±2.6 to 51.0±1.9 mg/dl, $p<0.05$). HDL PL content increased in patients with normal and high TG concentrations by 4.5 and 13.4%, respectively (Table 2).

Simvastatin and fenofibrate changed PL composition of HDL particles in patients with normal and high plasma TG concentrations (Table 2). The relative content of PC increased in patients of both groups. The relative content of PE increased in patients of both groups treated with simvastatin and in patients with hypertriglyceridemia receiving fenofibrate. The content of lysophosphatidylcholine decreased in all patients, while the content of sphingomyelin decreased only in patients treated with simvastatin (Table 2). The content of cardiolipin remained unchanged.

Since PL of TG-rich lipoproteins are transported to HDL during lipolysis, and peripheral lipoprotein lipase and, especially, hepatic lipase possess phospholipase activity, PL composition reflects the intensity of processes catalyzed by these enzymes. PC and PE are good substrates for hepatic lipase and their contents after passage through the liver decrease [11].

Studies of PL composition in patients with different TG concentrations showed that the contents of HDL PL, HDL PC, and HDL PE decreased in patients with hypertriglyceridemia and low content of HDL CH. Our results are consistent with published data on suppressed lipoprotein lipolysis and increased activity of hepatic lipase in patients with high VLDL and low HDL contents [10].

Hypolipidemic preparations used in our study as modifiers of lipoprotein content produce different ef-

TABLE 1. Lipid Content and PL Composition of HDL in Coronary Patients with Normal and Increased Plasma TG Concentrations ($M\pm m$)

Parameter	TG content, mg/dl	
	<180 (n=32)	≥180 (n=21)
CH, mg/dl	277.0±6.5	280.0±6.4
TG, mg/dl	109.0±5.5	232.0±14.4
HDL CH, mg/dl	51.0±1.6	44±2*
HDL PL, mg/dl	126±5	107.0±6.1*
HDL PL composition, %		
phosphatidylcholine	70.6±1.2	66±2**
lysophosphatidylcholine	12.6±0.8	16.7±1.3*
sphingomyelin	10.5±0.6	11.4±0.7
phosphatidylethanolamine	3.9±0.4	2.8±0.3**
cardiolipin	2.40±0.28	3.10±0.46

Note. *$p<0.01$ and **$p<0.05$ compared to patients with normal TG content (Student's t test).

TABLE 2. Lipid Content and PL Composition of HDL in Patients with Normal and Increased Plasma TG Concentrations before and after Treatment with Hypolipidemic Drugs (*M*±*m*)

Parameter	Simvastatin		Fenofibrate	
	TG<180 mg/dl (*n*=18)	TG≥180 mg/dl (*n*=8)	TG<180 mg/dl (*n*=14)	TG≥180 mg/dl (*n*=13)
CH, mg/dl	267.0±9.3 / 196.0±6.9*	271.0±10.6 / 201±10**	290.0±7.8 / 225.0±9.9*	285±8 / 238.0±13.7***
TG, mg/dl	—	193.0±3.2 / 155.0±19.2***	110.0±8.6 / 77.0±10.7***	257.0±20.3 / 131±20*
HDL PL, mg/dl	138.0±6.5 / 153±5*	125.0±9.6 / 133.0±6.8***	111.0±5.8 / 116.0±5.4**	97.0±6.7 / 110.0±5.9*
HDL PL composition, %				
lysophosphatidylcholine	9.90±0.85 / 5.40±0.61*	12.50±1.31 / 7.40±0.92*	16.00±0.97 / 14.00±0.27*	18.60±1.48 / 13.40±0.92*
sphingomyelin	10.90±0.87 / 9.3±0.6*	11.9±1.2 / 10.20±0.79***	10.10±0.73 / 10.10±0.85	11.20±0.87 / 10.30±0.75
phosphatidylcholine	73.8±1.26 / 78.90±0.81*	70.90±2.19 / 76.30±1.21*	66.60±1.68 / 69.20±1.49*	63.0±2.6 / 68.4±1.7*
phosphatidylethanolamine	3.60±0.53 / 4.60±0.46*	2.70±0.49 / 3.90±0.56**	4.30±0.59 / 4.10±0.44	3.00±0.46 / 3.60±0.34**
cardiolipin	1.80±0.28 / 1.70±0.27	2.10±0.44 / 1.90±0.41	3.20±0.57 / 2.80±0.44	4.30±0.84 / 3.00±0.57

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to parameters before therapy (pairwise Wilcoxon test).

fects on lipolysis. Simvastatin activates LDL reception, which can somewhat intensify lipoprotein lipolysis via the feedback mechanism; however, the contribution of these changes is relatively low. Fenofibrate regulates expression of genes encoding proteins involved in the metabolism of TG-rich lipoproteins. In particular, it promotes lipolysis via direct activation of lipoprotein lipase synthesis and suppression of production of its major inhibitor apolipoprotein III [2]. Therefore, fenofibrate decreases the content of TG (in particular, during hypertriglyceridemia) and increases HDL CH and HDL PL concentrations. Accumulation of PC and PE in HDL indicates inhibition of lipolysis, which is probably related to suppressed activity of hepatic lipase. Simvastatin produces similar changes in PL composition of HDL in patients with hypertriglyceridemia, but did not increase HDL CH content. Therefore, functional state of HDL is improved. These data confirm our assumption that decreased hepatic lipase activity plays a key role in the positive modifications of HDL PL composition. These changes in PL composition of HDL (even without significant increase in HDL CH content) promote the reverse CH transport from peripheral tissues to the liver and, probably, potentiate the antiatherogenic effect of HDL.

Our findings indicate that differences in PL composition of HDL particles in patients with low plasma TG content, as well as the changes in this parameter after reduction of TG concentration induced by hypolipidemic preparations, are related to the inhibition of hepatic lipase, rather than to modulation of lipoprotein lipolysis.

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