Impaired Antiatherogenic Function of High-Density Lipoproteins in the Presence of Various Risk Factors for Coronary Heart Disease

A. P. Serdyuk, I. N. Ozerova, T. V. Kamyshova, N. V. Perova, and V. A. Metel'skaya

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We studied acception of cholesterol from Fu5AH hepatoma cells by blood serum from subjects with normal level of high-density lipoprotein cholesterol and hyperlipidemia (alone or in combination with other risk factors for coronary heart disease). Cholesterol-binding activity of high-density lipoproteins decreased in subjects with hyperlipidemia alone or in combination with excess body weight and/or arterial hypertension. Impairment of high-density lipoprotein activity was associated with changes in their phospholipid composition.

Key Words: *high-density lipoproteins; cholesterol; reverse cholesterol transport; risk factors for ischemic heart disease*

Clinical and epidemiological studies demonstrated an independent negative correlation between the content of high-density lipoprotein (HDL) cholesterol in the blood and CHD-associated morbidity and mortality [10,13]. Low concentration of HDL cholesterol is often associated with other CHD risk factors, including hyperlipidemia (HLP), arterial hypertension (AH), and excess body weight [12]. The major function of HDL is reverse transport of cholesterol, which is a key mechanism of the regulation of serum cholesterol [8,14,15]. Disturbances in reverse cholesterol transport (*i.e.* cholesterol transport from peripheral tissues to liver and further excretion from the body [8,11]) lead to atherogenic shifts in serum lipid composition, which results in the development of atherosclerosis and its clinical manifestations.

Acception of excess cholesterol from cells to HDL is the initial stage of reverse cholesterol transport. Our previous studies showed that in patients with low concentration of HDL cholesterol this process in impaired compared to subjects with normal level of blood HDL cholesterol [2]. It is interesting to evaluate whether other metabolic risk factors of CHD (*e.g.*, isolated or combined HLP) can impair cholesterol binding from cells. Here we studied the efficiency of acception of cell cholesterol in subjects with normal level of HDL cholesterol and HLP (alone or in combination with excess body weight and/or AH).

MATERIALS AND METHODS

The study included subjects selected from 0.1% random representative sample of Moscow men (35-64 years). The study was performed within the framework of the Epidemiological Situation Monitoring Program (State Research Center for Preventive Medicine) to estimate the distribution of risk factors of CHD. Five groups with normal level of HDL cholesterol were selected: absence of CHD risk factors (control, n=8); HLP (n=12); HLP and excess body weight (n=5); HLP and AH (n=7); and HLP, excess body weight, and AH (n=26). Selection criteria corresponded to the European Policy Statement on the Prevention of CHD in clinical practice [6]. The HLP group

Department of Metabolic Disorders, State Research Center for Preventive Medicine, Russian Ministry of Health, Moscow. ***Address for correspondence:*** aserdyuk@gnicpm.ru. A. P. Serdyuk

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included patients with a cholesterol level≥190 mg/dl. The excess body weight group included patients with a body weight index≥25 kg/m^2. The AH group included patients with blood pressure≥140/90 mm Hg. Normal level of blood HDL cholesterol was above 40 mg/dl. The blood was taken from the cubital vein in the morning from fasting subjects. The contents of total cholesterol, triglycerides, and HDL cholesterol were measured enzymatically after precipitation of apo-B-containing lipoproteins with sodium phosphotungstate in the presence of magnesium chloride. The measurements were performed on an Airone 200 automatic analyzer using Human diagnostic kits. HDL phospholipids were analyzed in the supernatant after precipitation of serum LDL. Phospholipids were extracted by the method of Folch and separated into individual classes by means of thin-layer chromatography using glass plates coated with silica gel in two systems [1]. The concentration of phospholipids belonging to various classes was estimated by phosphorus content after mineralization with hydrazine hydrochloride and expressed as percentage of total HDL phospholipid concentration. Binding of cell cholesterol was studied using Fu5AH rat hepatoma cells [7]. The efficiency of binding was estimated by incubation of 5% serum samples with [^{3}H]-cholesterol-labeled cells at 37°C for 3 h. We calculated the amount of radioactive cholesterol released into the incubation medium. Intergroup differences were estimated by unpaired Student's *t* test at $p<0.05$.

RESULTS

The selected subjects of groups 1, 2, 3, 4, and 5 did not differ by age (50.1±2.8, 49.2±3.0, 50.6±4.0, 53.2±3.1, and 50.7±1.6 years, respectively). Triglyceride content was high in patients with HLP and excess body weight and/or AH (Table 1). The patients did not differ by HDL cholesterol concentration. Cholesterol acception from Fu5AH cells to blood serum from patients with HLP alone or in combination with excess body weight and/or AH was decreased by 11-15% compared to the control (Table 1). These data indicate that HLP alone or in combination with other metabolic risk factors of CHD is associated with impaired cholesterol acception from cells (stage I of reverse cholesterol transport).

Cholesterol-binding activity of HDL decreased in patients of 4 groups despite similar HDL cholesterol concentration in the control group and all 4 HLP groups. Probably, pre-β_1 HDL concentration decreases in patients with HLP. Published data show that pre-β_1-migrating HDL containing apoAI serve as the main acceptor of cell cholesterol [2,5]. Impaired antiatherogenic function of HDL in patients with HLP not differing in HDL cholesterol concentration attests to changes in quantitative characteristics of these particles. Antiatherogenic function of HDL is determined not only by the amount of these particles in the blood, but also by chemical composition of components in their surface monolayer (*e.g.*, phospholipids) [9]. The ratio between individual phospholipids in the surface monolayer of HDL determines its fluidity and, therefore, functional activity of these particles [9]. The phospholipid composition of HDL from patients with HLP differed from the control (Table 1). Cardiolipin content in HDL increased 2-fold in patients with HLP. No intergroup differences were revealed in the concentration of HDL lysophosphatidylcholine and phosphatidylethanolamine. Lysophosphatidylcholine concentration only tended to increase in patients with risk factors of CHD. In patients of various groups sphingomyelin content tended to increase (significant differences in the HLP group), while HDL phosphatidylcholine concentration decreased (significant differences in patients with HDL alone or in combination with excess body weight and AH). The phosphatidylcholine/sphingomyelin ratio in HDL tended to decrease in patients with CHD risk factors. A significant decrease in this parameter was revealed in patients with HLP alone or in combination with HLP and AH. Viscosity of carbohydrate domains in sphingomyelin is higher than that in phosphatidylcholine. The decrease in the phosphatidylcholine/sphingomyelin ratio reflects decreased membrane fluidity [9]. Phospholipids differ in the ability to form complexes with cholesterol. It should be emphasized that only cholesterol bound to phosphatidylcholine can be a substrate for blood lecithin cholesterol acyltransferase [3,9]. Sphingomyelin decreases lecithin cholesterol acyltransferase activity [4], which greatly affects cholesterol transporting function of HDL. The observed decrease in the phosphatidylcholine/sphingomyelin ratio in patients with CHD risk factors probably contributes to less efficient acception of cholesterol from cell membranes and lower degree of blood cholesterol esterification.

Our results suggest that the initial stage of reverse cholesterol transport (cholesterol transfer from cells to HDL) is impaired in patients with isolated or combined HLP. The decrease in cholesterol-binding activity of HDL in patients with HLP as well as in HLP in combination with excess body weight and/or AH can be related to changes in the phospholipid composition of these particles that determines fluidity of their surface layer (*e.g.*, decrease in the phosphatidylcholine/sphingomyelin ratio). Changes in the phospholipid composition in patients with HLP alone or in combination with metabolic risk factors of CHD can impair further stages of reverse cholesterol transport,

TABLE 1. Blood Lipids and Cholesterol Binding from Fu5AH Hepatoma Cells (*M*±*m*)

Parameter	Group				
	Control	HLP	HLP and excess body weight	HLP and AH	HLP, excess body weight, and AH
Total cholesterol, mg/dl	173.5±4.8	246.5±9.3***	259.2±22.1***	260.8±15.9***	248.5±9.0***
Triglycerides, mg/dl	66.5±8.5	117.1±32.1	152.9±41.9***	135.0±12.5*	160.9±16.6***
HDL cholesterol, mg/dl	49.8±4.1	54.9±4.9	51.0±3.2	46.8±4.7	45.6±2.0
HDL phospholipids, %					
lysophosphatidylcholine	10.80±0.73	12.80±1.05	12.50±1.49	12.70±1.97	12.50±0.58
sphingomyelin	11.30±0.42	13.6±0.6*	12.20±2.26	12.40±1.06	12.70±0.61
phosphatidylcholine	74.10±1.03	69.80±1.33**	71.2±3.5	70.40±1.65	70.70±0.88*
phosphatidylethanolamine	2.90±0.41	2.60±0.31	3.10±0.37	3.00±0.38	2.90±0.26
cardiolipin	0.90±0.19	1.8±0.3***	1.00±0.34	1.50±0.39	1.40±0.17
phosphatidylcholine/ sphingomyelin	6.60±0.26	5.25±0.28**	5.39±0.60	4.95±0.65**	5.93±0.32
Acception of cell cholesterol, %	16.95±0.22	15.09±0.34**	14.45±0.96*	14.76±0.54*	14.78±0.22***

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to the control.

i.e. cholesterol esterification in the plasma and transfer of cholesterol esters from HDL to other classes of lipoproteins and into the liver.

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