4. M. Burstein, H. R. Scholinick, and R. Morfin, *J. Lipid Res.*, **11**, 583-595 (1970).
5. M. Chauton, Y. Latron, F. Anfonso, *et al.*, *Ibid.*, **34**, 101-110 (1993).
6. H. Esterbauer and M. Kotheneder, *Fett. Wiss. Technol.*, **91**, 316-324 (1991).
7. H. H. Fei, J. A. Berliner, F. Parhami, and T. A. Drake, *Arterioscler. Thromb.*, **13**, 1711-1717 (1993).
8. J. Golley, J. Schenci, P. Schollmeyer, and C. Wanner, *Hypertension*, **21**, 315-321 (1993).
9. R. F. Itzahaki and D. M. Gill, *Anal. Biochem.*, **9**, 401-410 (1964).
10. F. T. Lindgren, *Analysis of Lipids and Lipoproteins*, E. G. Perkins (Ed.), Champagne (1975), pp. 204-224.
11. F. Lupu, I. Danaricu, and N. Simionescu, *Atherosclerosis*, **67**, 127-142 (1987).
12. R. O. Recknagel R.O. and E. A. Glende, *Methods Enzymol.*, **105**, 331-337 (1984).
13. U. P. Steinbrecher, M. Lougheed, W. C. Kwan and M. Dirks, *J. Biol. Chem.*, **264**, 15216-15223 (1989).
14. S. Steinbrecher, H. Zhang, and M. Lougheed, *Free Radic. Biol. Med.*, **9**, 155-168 (1990).
15. M. Uchiyama and M. Michara, *Anal. Biochem.*, **86**, 271-278 (1978).

Lipid Composition of Multiple Modified (Desialylated) Low-Density Lipoproteins

V. V. Tertov, V. V. Kaplun, I. A. Sobenin, and A. N. Orekhov

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It is shown that the lipid composition of desialylated low-density lipoproteins differs considerably from that of native (sialylated) lipoproteins. Desialylated lipoproteins have a lower content of fat-soluble vitamins and a higher *in vitro* oxidizability.

Key Words: *atherosclerosis; desialylated low-density lipoproteins; lipids*

Accumulation of lipids by intimal aortic cells is one of the main manifestations of atherosclerosis. It has been generally recognized that modified low density lipoproteins (LDL) circulating in human blood are an important atherogenic factor. We have detected modified LDL with a reduced content of sialic acid (desialylated LDL, DLDL) in human blood [5] and developed a method for their isolation [9]. It was demonstrated that DLDL but not sialylated LDL (SLDL) induce lipid accumulation in human aortic intimal cells, i.e., DLDL are atherogenic. In this study we compared the lipid composition of SLDL and DLDL.

MATERIALS AND METHODS

Pooled plasma of healthy subjects (24 males and 6 females aging 33-49 years) and of patients with ischemic heart disease and angiographically documented coronary atherosclerosis (24 males and 6 females aging 28-48 years) was used. None of the individuals had a history of diabetes mellitus or arterial hypertension. Low-density lipoproteins (1.019-1.063 g/ml) were isolated by ultracentrifugation [4]. DLDL and SLDL were separated by affinity chromatography on ricin-agglutinin (RCA 4120) agarose [9].

Lipids from cells and LDL were extracted as described [2] and [1], respectively. The total cholesterol content was determined by the method [7] using a Boehringer Mannheim kit.

Neutral lipids were separated by thin-layer chromatography with the use of two solvent systems: benzene:diethyl ether:ethanol:acetic acid (50:40:2:0.2, v/v) and n-hexane:diethyl ether:acetic acid (90:10:1, v/v). The contents of individual lipids were measured by scanning densitometry [6].

Phospholipids were separated using a methylacetate:n-propanol:chloroform:methanol:0.25% KCl (25:25:25:10:9, v/v) mixture. The phospholipid con-

Cardiology Research Center, Russian Academy of Medical Sciences, Moscow

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tent was measured by scanning densitometry of by colorimetric determination of lipid phosphorus [10].

The hydroxysterol content was determined with the use of a kit (Boehringer Mannheim) for enzyme determination of cholesterol after alkaline hydrolysis of lipids and their separation by thin-layer chromatography in a hexane:acetone system (3:1, v/v).

The sialic acid content was determined as described elsewhere [8]. The levels of thiobarbituric acid-reactive substances (TBARS) in LDL preparations were measured by the method of Yagi [11]. The contents of vitamins E and A were determined as described [3].

RESULTS

The content of neutral lipids in SLDL and DLDL of healthy subjects and patients is given in Table 1. The contents of the major neutral lipids (triglycerides and free and esterified cholesterol) were practically the same in pooled LDL of healthy subjects and patients. Meanwhile, the contents of monoglycerides and free fatty acids were higher in pooled LDL of patients (Table 1).

The levels of neutral lipids in SLDL and pooled LDL of healthy subjects were practically the same (Table 1). In DLDL of healthy subjects, the content of free and esterified cholesterol was 30-40% lower and that of free fatty acids monoglycerides 1.5-fold higher than in SLDL and pooled LDL.

The content of triglycerides and free and esterified cholesterol in patients' SLDL was 20-25% higher than in pooled lipoproteins (Table 1). The content of triglycerides and of free and esterified cholesterol in patients' DLDL was 1.5- to 2-fold lower than in pooled LDL (Table 1). However, the level of free fatty acids, mono-, and diglycerides in them was 3- to 5-fold higher than in pooled LDL or SLDL.

Table 2 shows the content of major phospholipids in LDL of healthy subjects and patients. Pooled LDL of patients had a lower content of phosphatidylethanolamine and higher content of lysophosphatidylcholine compared with that in pooled LDL of healthy subjects. The lipid composition of SLDL of healthy subjects was practically the same as that of the pooled LDL and SLDL of patients (Table 2).

TABLE 1. Content of Neutral Lipids in SLDL and DLDL in the Plasma of Healthy Subjects and Patients with Ischemic Heart Disease

Fraction	Lipid content, μg/mg protein					
	esterified cholesterol	cholesterol	triglycerides	diglycerides	monoglycerides	free fatty acids
Healthy subjects						
Pooled LDL	1956±19	587±17	260±19	16±1	24±1	58±3
SLDL	2055±132	628±29	268±18	15±1	29±2	58±2
DLDL	1507±58	427±14	206±7	15±1	39±1	79±3
Patients						
Pooled LDL	1793±102	521±29	269±15	19±1	46±3	98±3
SLDL	2215±74	638±27	301±27	15±1	27±2	53±2
DLDL	1412±30	359±38	196±12	25±1	82±4	196±2

TABLE 2. Content of Phospholipids in SLDL and DLDL in the Plasma of Healthy Subjects and Patients with Ischemic Heart Disease

Fraction	Phospholipid content, μg/mg protein					
	phosphatidylcholine	lysophosphatidylcholine	sphingomyelin	phosphatidylethanolamine	phosphatidylinositol	phosphatidylserine
Healthy subjects						
Pooled LDL	897±30	53±4	416±10	42±2	37±2	21±1
SLDL	905±89	51±3	408±14	34±3	35±2	23±2
DLDL	752±36	71±3	402±15	26±2	30±3	19±1
Patients						
Pooled LDL	846±41	79±5	374±21	32±1	35±3	19±1
SLDL	1105±96	56±4	407±17	41±2	37±3	24±2
DLDL	673±37	103±4	308±11	19±1	29±3	17±2

TABLE 3. Content of TBARS and Vitamins A and E in SLDL and DLDL of Healthy Subjects and Patients with Ischemic Heart Disease

Fraction	TBARS, pmol/mg protein		Hydroxysterols, μg/mg protein	Vitamins, μg/mg protein	
	control	+10 μM $CuCl_2$		A	E
Healthy subjects					
Pooled LDL	186±13	2485±104	20±2	0.23±0.1	4.5±0.5
SLDL	179±18	2496±152	19±1	0.25±0.2	4.3±0.3
DLDL	215±22	4956±208	34±3	0.19±0.1	3.5±0.2
Patients					
Pooled LDL	223±21	5079±321	41±3	0.18±0.1	3.1±0.2
SLDL	179±18	2846±280	26±3	0.21±0.2	3.9±0.3
DLDL	215±22	8496±307	78±4	0.14±0.1	2.1±0.2

DLDL of healthy subjects had a lower content of phosphatidylcholine and higher content of lysophosphatidylcholine compared with pooled and sialylated LDL.

The phospholipid composition of SLDL of healthy subjects and patients proved to be practically the same. The content of phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin in patients' DLDL was 1.5- to 2.0-fold lower than in SLDL. On the other hand, the lysophosphatidylcholine content of patients' DLDL was twice as that of SLDL (Table 2). The TBARS content in pooled LDL of healthy subjects and patients was virtually the same (Table 3). SLDL and DLDL did not differ considerably in the TBARS content. Incubation of LDL in the presence of 10^{-5} M Cu^{2+} led to a marked increase in the TBARS content (Table 3). It is noteworthy that the increase in the TBARS level in patients' LDL was twice as that in LDL of healthy subjects. However, the content of TBARS in copper-oxidized DLDL of healthy subjects was twice as that in oxidized SLDL. Patients' DLDL had the highest oxidizability.

Four major hydroxysteroids were identified in LDL by thin-layer chromatography: 7-keto-, 5,6-diene-, 7-hydroxy-, and 25-hydroxysteroid. The total content of hydroxysteroids in LDL of healthy subjects and patients is given in Table 3. The hydroxysteroid content in DLDL (both of healthy subjects and patients) was 2- to 4-fold higher than that in SLDL.

The content of fat-soluble vitamins (A and E) was much lower in DLDL than in native LDL (Table 3), which may account for the higher *in vitro* oxidizability of DLDL.

Thus, we have shown that DLDL markedly differ from native (sialylated) LDL in lipid composition, the differences being more pronounced in LDL of patients with ischemic heart disease.

Desialylated LDL have a lower lipid content and, consequently, higher density and smaller size in comparison with SLDL. The loss of fat-soluble vitamins results in a higher oxidizability of these LDL. The mechanism underlying these modifications is unclear. Further investigation of this problem is important for better understanding of modifications that render LDL atherogenic.

REFERENCES

1. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
2. A. Hara and N. S. Radin, *Anal. Biochem.*, **90**, 420-426 (1978).
3. S. A. Hashim and G. M. Schuttringer, *Am. J. Nutr.*, **19**, 137-147 (1966).
4. F. T. Lindgren, in: *Analysis of Lipids and Lipoproteins,* New York (1975), pp. 205-224.
5. A. N. Orekhov, V. V. Tertov, D. H. Muhin, *et al.*, *Biochem. Biophys. Res. Commun.*, **162**, 206-211 (1989).
6. A. N. Orekhov, V. V. Tertov, I. D. Novikov, *et al.*, *Exp. Mol. Pathol.*, **42**, 117-137 (1985).
7. J. Siedel, E. O. Hagele, J. Liegenhorn, *et al.*, *Clin. Chem.*, **29**, 1075-1080 (1983).
8. L. Svennerholm, *J. Neurochem.*, **1**, 45-53 (1959).
9. V. V. Tertov, I. A. Sobenin, A. G. Tonevitsky, *et al.*, *Biochem. Biophys. Res. Commun.*, **167**, 1122-1127 (1990).
10. V. E. Vaskovsky, E. Y. Kostetsky, I. M. Vasendin, *et al.*, *J. Chromatogr.*, **114**, 129-141 (1975).
11. K. Yagi, *Methods Enzymol.*, **105**, 328-333 (1984).