

COMPARATIVE CHARACTERISTICS OF FATTY-ACID  
COMPOSITION OF LIPOPROTEINS FROM HUMAN  
BLOOD PLASMA AND AORTIC WALL

A. D. Denisenko and A. N. Klimov\*

UDC 612.123/.124:612.397.23

The fatty-acid composition of lipid fractions in lipoproteins of very low, low, and high density from blood plasma and the aortic wall of patients with atherosclerosis was investigated. A close similarity was found in the fatty-acid composition of phospholipids, triglycerides, and cholesterol esters in all classes of lipoproteins from the vascular wall and blood plasma. The composition of the fatty acids of the fractions was very similar in all classes of lipoproteins. Lipids from the vascular wall not included in the composition of lipoproteins differed considerably in their fatty-acid composition from lipids isolated from lipoproteins: the content of unsaturated fatty acids in the lipids of the aortic wall was much smaller than in lipoproteins.

KEY WORDS: lipoproteins; aorta; atherosclerosis; fatty-acid composition of lipids.

The human vascular wall was shown previously to contain the same spectrum of lipoproteins as the blood serum (pre- $\beta$ -,  $\beta$ -, and  $\alpha$ -lipoproteins); the  $\beta$ - and  $\alpha$ -lipoproteins of the blood serum and aortic wall, moreover, are immunologically identical [1]. These findings are evidence of the plasma origin of the lipoproteins of the vascular wall. At the same time, evidence has been published that the aortic wall itself can synthesize lipoproteins [7] and that the phospholipid composition of lipoproteins of the vascular wall differs from its composition in lipoproteins of the blood plasma [8], although their protein parts are immunologically identical.

Accordingly it was decided to compare the fatty-acid composition of lipoproteins from the blood plasma and vascular wall.

## EXPERIMENTAL METHOD

Blood and the aorta from 3 men and 3 women dying from various causes, age 50-65 years old, were investigated. The material was taken not more than 24 h after death. Lipoproteins of very low, low, and high density (VLDL, LDL, and HDL) [6] were isolated by preparative ultracentrifugation from the blood plasma and the tissue fluid obtained from the atherosclerotic aortic wall [1]. Lipids from lipoproteins and from the minced aortic wall after isolation of the tissue fluid from it were extracted by Folch's method and fractionated by thin-layer chromatography in a system of petroleum ether-diethyl ether-acetic acid (90:10:1). After elution from silica gel, the phospholipids, triglycerides, and cholesterol esters were hydrolyzed with 2 N NaOH solution in methanol and the free fatty acids were methylated with the aid of a 14% solution of  $\text{BF}_3$  in methanol. Gas-liquid chromatography was carried out on the "Tsvet" model 4-67 chromatograph using a flame ionization detector. Celite-22 (80-100 mesh) in the stationary phase with polyethylene glycol succinate (20%) was used as the solid carrier; the carrier gas was nitrogen (velocity 2 liters/h). The length of the column was 3 m, the temperature of the vaporizer 250°C, and the column temperature 195°C.

\* Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Lipid Metabolism, Department of Atherosclerosis, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 79, No. 6, pp. 44-47, June, 1975. Original article submitted May 17, 1974.

TABLE 1. Fatty-Acid Composition of Cholesterol Esters of Lipoproteins and Aortic Tissue (in %);  $M \pm m$

Fatty acids	VLDL		LDL		HDL		Aortic tissue after separation of tissue fluid
	serum	tissue fluid	serum	tissue fluid	serum	tissue fluid	
12:0	3,2±0,4	2,8±0,4	2,8±0,4	2,5±0,4	3,3±0,4	4,0±0,4	1,8±0,4
14:0	2,0±0,3	2,1±0,3	3,0±0,3	3,7±0,4	2,2±0,3	2,9±0,4	2,0±0,3
16:0	12,8±1,5	11,5±1,4	12,7±1,3	11,1±1,5	10,9±1,6	11,8±1,5	18,3±1,9
16:1	4,2±0,8	5,7±0,6	5,5±0,6	5,7±0,7	5,9±0,7	6,1±0,6	7,3±0,7
18:0	4,0±0,5	4,8±0,5	4,2±0,5	5,4±0,6	5,5±0,6	4,8±0,6	3,6±0,5
18:1	21,8±2,1	22,6±2,0	24,3±2,2	22,5±2,5	21,7±2,0	20,3±2,0	35,1±3,1
18:2	39,8±3,0	39,2±3,0	38,1±3,1	37,9±3,1	38,9±3,3	39,9±3,0	25,3±3,3
18:3	5,9±1,0	4,5±0,9	4,3±0,5	4,6±1,0	5,3±0,9	4,1±0,7	3,1±1,0
20:4	6,3±0,9	6,8±1,0	5,1±0,6	6,6±0,7	6,3±0,7	6,1±0,6	3,5±0,6
Saturated	22,0±1,9	21,2±2,0	22,7±1,8	22,7±2,0	21,9±1,9	23,5±2,3	25,7±1,9
Monounsaturated	26,0±2,1	28,3±2,3	29,8±2,5	28,2±2,4	27,8±2,0	26,4±2,7	42,4±3,8
Polyunsaturated	52,0±2,5	50,5±3,5	47,5±4,0	49,1±3,7	50,5±3,7	50,1±4,1	31,9±3,7

TABLE 2. Fatty-Acid Composition of Triglycerides of Lipoproteins and Aortic Tissue (in %);  $M \pm m$

Fatty acids	VLDL		LDL		HDL		Aortic tissue after separation of tissue fluid
	serum	tissue fluid	serum	tissue fluid	serum	tissue fluid	
12:0	2,3±0,5	1,3±0,4	1,9±0,4	2,0±0,4	1,0±0,5	1,2±0,3	1,0±0,4
14:0	2,0±0,4	1,0±0,4	3,0±0,6	2,2±0,5	1,8±0,4	1,6±0,3	1,9±0,4
16:0	25,4±2,1	26,3±2,1	27,2±2,2	26,9±2,0	27,2±2,3	26,6±2,2	28,1±2,5
16:1	3,7±0,5	5,0±0,6	2,7±0,4	3,1±0,5	2,7±0,4	3,3±0,5	3,5±0,5
18:0	10,9±0,9	10,1±0,9	11,3±0,8	10,3±1,0	10,3±1,0	11,1±0,9	14,1±0,9
18:1	35,9±2,7	37,0±2,9	34,0±2,6	32,2±3,0	35,5±2,7	34,3±2,9	36,6±3,0
18:2	12,0±1,0	13,6±1,1	12,5±1,1	14,1±1,4	13,3±1,2	14,1±1,2	6,7±1,1
18:3	4,4±0,7	3,8±0,6	5,0±0,5	6,7±0,6	5,2±0,5	4,1±0,6	4,0±0,6
20:4	3,4±0,5	2,0±0,4	2,4±0,4	2,5±0,4	3,2±0,5	3,7±0,5	4,1±0,7
Saturated	40,6±2,1	38,7±2,2	43,4±2,0	41,4±2,0	40,3±2,0	40,5±2,0	45,1±2,0
Monounsaturated	39,6±2,0	42,0±2,0	36,7±1,9	35,3±1,9	38,0±1,8	37,6±1,8	40,1±1,8
Polyunsaturated	20,8±1,5	19,4±1,4	19,9±1,4	23,3±1,5	21,7±1,4	21,9±1,5	14,8±1,2

TABLE 3. Fatty-Acid Composition of Phospholipids of Lipoproteins and Aortic Tissue (in %);  $M \pm m$

Fatty acids	VLDL		LDL		HDL		Aortic tissue after separation of tissue fluid
	serum	tissue fluid	serum	tissue fluid	serum	tissue fluid	
12:0	2,8±0,3	3,0±0,4	2,1±0,4	2,1±0,4	1,8±0,3	1,8±0,3	1,2±0,3
14:0	1,8±0,3	2,8±0,4	1,8±0,3	2,0±0,3	2,1±0,4	2,0±0,4	1,0±0,3
16:0	30,6±1,7	33,1±1,6	33,0±1,5	32,5±1,6	32,3±1,5	31,7±1,6	34,0±1,5
16:1	5,4±0,5	5,3±0,5	3,5±0,5	4,1±0,4	5,2±0,5	4,1±0,4	2,6±0,4
18:0	16,7±1,0	17,3±0,9	17,7±0,9	16,9±1,1	15,7±1,1	16,1±1,0	22,4±1,2
18:1	16,9±1,1	16,7±1,1	17,3±1,0	17,0±1,2	17,3±1,1	16,9±1,1	17,1±1,2
18:2	20,3±1,3	18,3±1,2	18,7±1,2	19,3±1,3	18,7±1,2	19,0±1,2	15,0±1,3
18:3	2,3±0,4	1,3±0,3	2,5±0,4	2,7±0,4	2,8±0,4	3,1±0,4	2,9±0,4
20:4	3,2±0,5	2,2±0,4	3,4±0,4	3,4±0,5	3,9±0,5	5,3±0,6	3,7±0,5
Saturated	51,9±2,4	56,2±2,5	54,6±2,3	53,5±2,4	51,9±2,4	51,6±2,3	58,4±3,1
Monounsaturated	21,3±1,2	22,0±1,2	20,8±1,2	21,1±1,0	22,5±1,2	21,0±1,1	19,7±2,2
Polyunsaturated	25,8±1,5	21,8±1,6	24,6±1,3	25,4±1,2	25,4±1,2	27,4±1,3	21,6±1,7

## EXPERIMENTAL RESULTS AND DISCUSSION

The fatty-acid composition of cholesterol esters of the lipoproteins from blood serum and aortic tissue fluid (Table 1) was virtually identical. Linoleic acid (18:2) was the chief fatty acid of the cholesterol esters and accounted for about 37% of the total fatty acids of the esterified lipoprotein cholesterol. The fatty-acid composition of cholesterol esters in the vascular wall but outside the lipoprotein particles differed

considerably from that in all classes of lipoproteins. A high content of palmitic (16:0) and, in particular, of oleic (18:1) acids and a lower content of linoleic (18:2) and arachidic (20:4) acids was found in the cholesterol esters of the aorta after separation of the tissue fluid from it ( $P < 0.05$ ). Other workers have observed similar proportions in the fatty-acid composition of the cholesterol esters of the blood serum and vascular wall [3, 9].

The fatty-acid composition of the triglycerides (Table 2) isolated from VLDL, LDL, and HDL of the tissue fluid and blood serum also was very similar. The chief fatty acid was oleic (18:1), which accounted for about 35% of the total fatty acids of the triglycerides. Comparison of the fatty-acid composition of the triglycerides of the aortic tissue and lipoproteins showed a considerable difference in the content of stearic (18:0) and linoleic (18:2) acids ( $P < 0.05$ ).

Examination of the fatty-acid composition of the phospholipids of lipoproteins of the blood serum and aortic tissue fluid (Table 3) revealed their great similarity. Palmitic acid (16:0) accounted for about 32% of the total fatty acids and was the chief acid of the phospholipids. Phospholipids of the aortic tissue after separation of the tissue fluid from it contained more stearic (18:0) and less linoleic (18:2) acids than the phospholipids of VLDL, LDL, and HDL of the blood serum and tissue fluid ( $P < 0.05$ ), also in agreement with data in the literature [3, 9].

Analysis of the results shows that the fatty-acid composition of the lipid fractions in the various classes of lipoproteins from the blood serum and aortic tissue fluid was very similar; meanwhile, each lipid fraction had its own strictly definite ratio between the various fatty acids. Data in the literature on this question are contradictory. Smith, for instance, found a considerable difference between the fatty-acid composition of cholesterol esters of VLDL and LDL [10], whereas other workers note the great similarity between the fatty-acid composition of all classes of blood serum lipoproteins [9] or even its complete identity [4].

The observed identity of the fatty-acid composition of lipoproteins from the blood serum and aortic tissue fluid is evidence of the plasma origin of the lipoproteins of the vascular wall. The immunologic identity of lipoproteins of the blood plasma and aortic wall was demonstrated previously [1, 11]. These facts suggest that the plasma lipoproteins penetrate into the vascular wall as a complete particle. This is confirmed by data on the penetration of lipoproteins with a double radioactive label into the aorta of rabbits with experimental atherosclerosis [2].

Comparative investigation of the fatty-acid composition of the vascular tissue lipids and lipoproteins showed a significant difference between them: aortic tissue lipids contain less unsaturated fatty acids than lipoproteins (mainly on account of linoleic acid). The considerable difference in the fatty-acid composition of lipoproteins and lipids of aortic tissue with atherosclerotic changes indicates high activity of the enzymes responsible for hydrolysis and synthesis of fatty acid esters in the vascular wall, evidently in connection with the increased synthesis of phospholipids and cholesterol esters in the aortic wall in atherosclerosis [5, 12].

#### LITERATURE CITED

1. A. N. Klimov, A. D. Denisenko, and E. Ya. Magracheva, *Atherosclerosis*, 19, 243 (1974).
2. A. N. Klimov, T. N. Lovyagina, and E. B. Ban'kovskaya, *Vopr. Med. Khimii*, 17, 434 (1972).
3. A. J. Day and M. L. Wahlquist, *Exp. Molec. Path.*, 13, 109 (1970).
4. W. S. Goodman and T. Shiratory, *J. Lipid Res.*, 5, 307 (1964).
5. S. Hashimoto, S. Dayton, and R. B. Alfin-Slater, *Life Sci.*, 12, 1 (1973).
6. R. J. Havel, H. A. Eder, and J. H. Bragton, *J. Clin. Invest.*, 34, 1345 (1955).
7. W. Hollander, *Exp. Molec. Path.*, 7, 248 (1967).
8. H. J. Kayden, B. C. Seegal, and K. S. Hsu, *J. Clin. Invest.*, 38, 1016 (1959).
9. T. D. Lawrie, S. G. McAlpine, B. M. Rifkind, et al., *Clin. Sci.*, 27, 89 (1964).
10. E. B. Smith, *J. Atheroscler. Res.*, 5, 224 (1965).
11. R. E. Tracy, E. B. Merchant, and V. C. Kao, *Circulat. Res.*, 9, 472 (1961).
12. D. B. Zilversmit, E. L. McCandless, P. H. Jordan, et al., *Circulation*, 23, 370 (1961).