Journal of Chromatography, 273 (1983) 180—186
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1542

Note

Gas—liquid chromatography profiling of intact lipids

Observation of differences between triglyceride structure of lipoproteins in type III and type IV hyperlipoproteinemia

J. SKOŘEPA*, V. KAHUDOVÁ, E. KOTRLÍKOVÁ, P. MAREŠ and H. TODOROVIČOVÁ

IVth Medical Clinic and Lipid Metabolism Laboratory, Faculty of Medicine, Charles University, Prague (Czechoslovakia)

In recent years there has been great interest in the study of the metabolism of twenty-carbon polyunsaturated fatty acids which are precursors of prostaglandins and leukotrienes. For these studies efficient chromatographic techniques have been used. Kuksis et al. [1] introduced in 1967 the gas chromatographic analysis of intact lipids. Smith et al. [2] used high-performance liquid chromatography for the study of heterogeneous triglyceridemia in animal models. Mareš and co-workers [3, 4] modified the original method of Kuksis for gas chromatographic profiling of human plasma intact neutral lipids in the nanogram range; the method allows further study of subtle changes in triglyceride composition of individual plasma lipoprotein classes [3, 4].

In the present study we have monitored the triglycerides of total plasma and the triglycerides of individual plasma lipoprotein classes. The purpose of the study was to compare the molecular weight distribution of triglycerides with the corresponding fatty acid profile. Triglyceride composition was studied in subjects suffering from heterogeneous triglyceridemia. They were given an experimental diet supplemented with marine fish rich in very-long-chain fatty acids.

METHODS

Two female patients were given a diet supplemented with mackerel 300—400 g/day for a period of five days. Patient No. 1, 53 years old, had familial dyslipoproteinemia type III. She has had persistent heterogeneous triglyceridemia since our first contact with her [5]. Patient No. 2, 59 years old, was an insulin-dependent diabetic and her diet was adapted to this conditions. She had

0378-4347/83/0000—0000/$03.00

secondary hyperlipoproteinemia type IV and intermittent heterogeneous triglyceridemia.

Venous blood was drawn for analysis after an overnight fast before starting the experiment and after five days of mackerel-supplemented diet; EDTA was used as anticoagulating agent in a concentration of 1 mg/ml. For lipoprotein separation a sample of blood was drawn the fifth day of the experiment, 4 h after the midday meal. Individual plasma lipoprotein classes [chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)] were isolated by preparative ultracentrifugation. The purity of the individual lipoprotein classes was checked by agarose gel electrophoresis.

Neutral lipid profile

Neutral lipids were extracted from 250 μl of plasma using 5 ml of extraction mixture composed of equal volume parts of chloroform, methanol and acetone. Polar lipids were removed by Florisil added to each plasma sample before the extraction. Lipids were analysed on a 0.5-m glass column packed with 3% OV-1 on Gas-Chrom Q 100—120 mesh under conditions already described [3].

Fatty acid analysis

Plasma lipids and lipoprotein fractions were extracted according to the procedure of Folch et al. [6]. Individual lipid classes (cholesteryl esters, triglycerides, and phosphatidylcholines) were separated by thin-layer chromatography on silica gel $HF_{254+366}$ plates (E. Merck, Darmstadt, G.F.R.). For the separation of cholesteryl ester and triglycerides, plates were developed in *n*-heptane—diethyl ether—acetic acid (85:15:1, v/v). Phosphatidylcholines were developed in chloroform—methanol—water (60:30:5, v/v). Separated lipid classes were detected under an UV lamp (366 nm) after the spraying with 0.01% 2,7-dichlorofluorescein in ethanol. Spots were scraped off and the lipids were isolated using the "dry column" technique. Cholesteryl esters and triglycerides were eluted three times with 2-ml portions of elution mixture composed of equal volume parts of chloroform and methanol. Phosphatidylcholines were eluted by the same technique using a mixture of chloroform—methanol—water—acetic acid (13:5:1:0.2, v/v). All lipid classes were transesterified using 0.5 *N* sodium methoxide and analyzed by gas—liquid chromatography (GLC) under the following conditions: gas chromatograph Perkin-Elmer Sigma 1, injector and detector temperature (250° C, oven programme from 120° C to 200° C (rate 2.5° C/min), flame ionization detector, attenuation 10 × 8, column 1.8 m × 2 mm I.D., glass, packed with 10% OV-275 (Supelco, Bellefonte, PA, U.S.A.) on Chromosorb W AW 80—100 mesh (Carlo Erba, Milan, Italy). For quantitative analysis an internal standard method was used and results were calculated by means of a Perkin-Elmer Sigma 10 Laboratory Data Station. The reproducibility of the results was checked using control samples prepared from pure compounds.

RESULTS

Fatty acid composition of plasma triglycerides

The fatty acid composition of whole plasma triglycerides after five days of mackerel-supplemented diet is shown in Table I. Twenty-carbon and 22 carbon polyenes and monoenes which are characteristic of mackerel oil were correspondingly higher after the diet. The effect of the diet was the same in both subject.

TABLE I

FATTY ACID COMPOSITION OF WHOLE PLASMA TRIGLYCERIDES BEFORE AND AFTER THE DIET

Results are expressed as mol%.

Fatty acid	Patient No. 1, dyslipoproteinemia type III		Patient No. 2, hyperlipoproteinemia type IV	
	Before	After	Before	After
14:0	1.8	2.5	1.5	1.6
16:0	27.7	17.8	31.2	24.9
16:1	5.0	3.6	5.6	4.3
18:0	2.1	2.6	2.2	2.9
18:1	46.1	29.0	48.2	35.2
18:2 (*n*—6)	13.6	9.6	8.8	8.2
18:3 (*n*—6)	0.4	0.2	0.2	0.3
18:3 (*n*—3)	0.7	1.2	0.6	0.9
20:0	0.2	0.1	0.3	0.1
20:1	0.3	3.3	0.3	1.4
20:3 (*n*—6)	0.2	0.1	0.2	0.1
20:4 (*n*—6)	1.0	2.1	0.5	1.2
20:5 (*n*—3)	0.2	11.9	<0.1	7.3
22:1	<0.1	1.6	<0.1	0.9
22:5 (*n*—3)	0.2	1.1	0.1	1.0
22:6 (*n*—3)	0.3	13.3	0.4	9.7
Totals of C_{20} fatty acids	1.9	17.5	1.3	10.2
Totals of C_{22} fatty acids	0.6	16.1	0.5	11.6

Fatty acid composition of lipoprotein triglycerides

Table II shows the fatty acid composition of plasma lipoprotein classes after the mackerel-supplemented diet. There is a considerable proportion of very-long-chain fatty acids in all lipoproteins with a decrease from low-density to high-density classes.

Molecular-weight profile of lipoprotein triglycerides

Fig. 1 demonstrates the molecular-weight profiles of neutral lipids isolated from individual lipoprotein classes. The patterns of triglyceride look more

TABLE II

FATTY ACID COMPOSITION OF PLASMA LIPOPROTEIN TRIGLYCERIDES AFTER THE DIET

Results are expressed as mol%.

Fatty acid	Patient No. 1, dyslipoproteinemia type III				Patient No. 2, hyperlipoproteinemia type IV			
	CM	VLDL	LDL	HDL	CM	VLDL	LDL	HDL
14:0	4.5	3.6	2.7	3.4	3.5	2.6	2.3	2.9
16:0	14.7	19.5	17.4	20.1	16.6	24.7	23.8	23.5
16:1	3.8	4.5	3.7	5.6	3.2	3.8	3.9	5.3
18:0	2.2	2.9	2.7	3.2	2.6	2.9	3.4	5.0
18:1	25.3	26.6	29.5	29.5	30.4	33.6	36.3	35.1
18:2 (*n*—6)	6.3	9.0	9.7	9.4	6.8	7.8	8.0	8.1
18:3 (*n*—6)	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
18:3 (*n*—3)	2.3	1.5	1.2	1.2	2.0	1.4	1.3	1.5
20:0	tr.	<0.1	<0.1	<0.1	tr.	0.2	0.2	0.3
20:1	12.2	4.5	3.6	3.4	8.9	3.4	2.9	2.7
20:3 (*n*—6)	0.2	0.2	0.1	0.1	0.2	0.1	0.1	<0.1
20:4 (*n*—6)	0.9	1.8	2.1	2.0	0.8	0.8	1.2	1.3
20:5 (*n*—3)	6.9	9.5	11.4	8.5	7.5	5.3	5.0	4.3
22:1	11.1	3.6	1.7	1.8	8.9	2.8	2.1	1.6
22:5 (*n*—3)	1.3	1.4	1.1	1.3	1.2	2.2	1.4	2.1
22:6 (*n*—3)	8.2	11.3	12.8	10.0	7.4	8.3	7.6	5.9
Totals of C_{20} fatty acids	20.2	16.0	17.4	14.2	17.5	9.8	9.4	8.6
Totals of C_{22} fatty acids	20.5	16.2	15.6	13.2	17.5	13.3	11.1	9.6

informative than the corresponding fatty acid profiles. From the digital equivalents in Fig. 2 it is evident that the carbon-number* distribution of chylomicron triglycerides was rather symmetrical; it was very close to the random distribution calculated from the compositon of the fatty acids. The carbon-number profile of VLDL triglyceride reveals a tendency to non-randomness and this trend was again more expressed in LDL triglycerides. The composition of HDL triglycerides showed evidence of non-random selection of triglyceride fatty acids. Higher-molecular-weight species in the HDL triglyceride profile were only minimal.

DISCUSSION

Heterogeneous triglyceridemia in human subjects is characterized by a relative elevation of higher-molecular-weight triglyceride species containing

*For cholesteryl esters the carbon number corresponds to the total number of carbon atoms, whereas for triglycerides it corresponds to the sum of the carbon atoms in the fatty acid moieties.

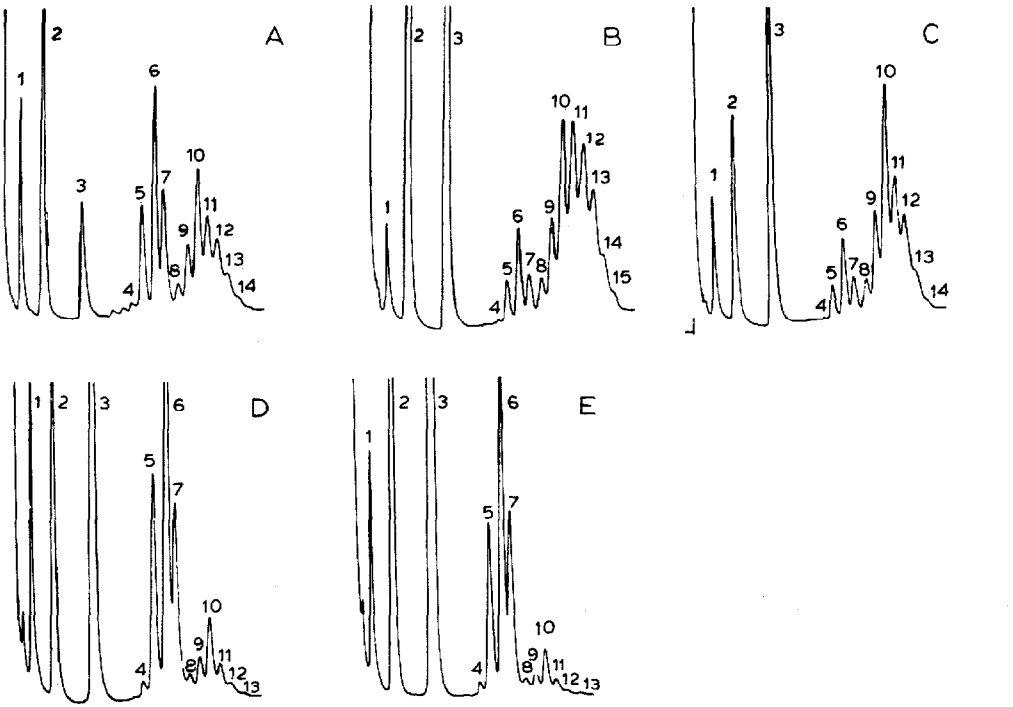

Fig. 1. Neutral lipid profiles of plasma and individual lipoprotein classes of the same subject. (A) Whole plasma; (B) chylomicrons; (C) VLDL; (D) LDL; (E) HDL. 1 = Cholesterol, 2, 3 = standards, 4—7 = cholesteryl esters with carbon numbers 41—47, 8—15 = triglycerides with carbon numbers 48—62. For analytical conditions see Methods section.

very-long-chain fatty acids. The anomaly is present in about 25% of hypertriglyceridemias but it can also be found in subjects with normal plasma triglyceride levels [5].

It is very easy to induce the condition with a diet rich in very-long-chain fatty acids as, for instance, contained in marine fish oils, but in some subjects the anomaly persists even if very-long-chain fatty acids are excluded from the diet as we have observed in our patient No. 1, who is an index member of a dyslipoproteinemia type III family referred to previously [5]. We have studied the effect of mackerel diet on the fatty acid composition and molecular-weight profile of triglycerides both in whole plasma and in isolated lipoprotein fractions.

Chylomicron triglycerides were characterized by a high proportion of very-long-chain triglyceride fractions with carbon numbers of 60, 62 and even 64. The triglyceride component of chylomicrons is formed in the intestinal mucosa, mostly from exogenous dietary fatty acids. Molecular-weight distribution is very close to the theoretical random distribution of fatty acids of triglyceride molecules. With increasing density of lipoprotein particles the effect of non-random selection of fatty acids for triglyceride molecule formation is more and more evident. In HDL triglycerides the proportion of high-molecular-weight species is minimal even if very-long-chain fatty acids are still present in high proportions.

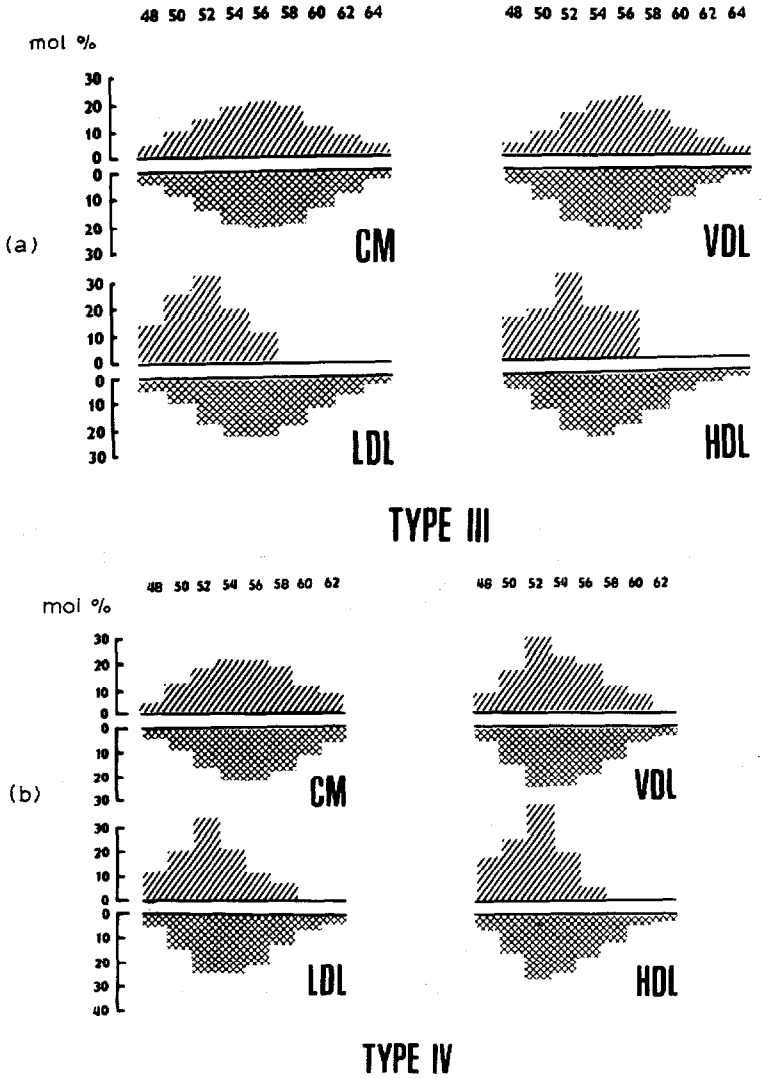

Fig. 2. Molecular-weight distribution of lipoprotein triglyceride components in type III and type IV hyperlipoproteinemia. Comparison of GLC-assessed values (above) with theoretical values calculated as random distribution of fatty acids in triglyceride molecules (below).

The essential divergence between fatty acid composition and molecular-weight profile of lipoprotein triglyceride species is most evident from geometrical representation in multidimensional euclidean space. Let us represent lipoprotein triglycerides as vectors calculated as molar per cent carbon number fractions in one case and as molar per cent fatty acids in the other case. Sets of angles among vectors in both systems were quite different. Projection of tips of vectors on the same plane generated a map of distances between lipoprotein triglyceride in both systems (Fig. 3). It is evident that the intact lipid profile was much more informative for discovering subtle differences among triglycerides composed of similar sets of fatty acids but in distinct non-random combinations.

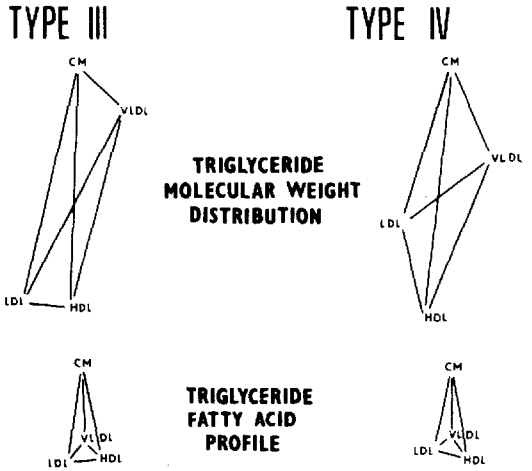

Fig. 3. Map of distances between lipoprotein triglyceride structure in type III and type IV hyperlipoproteinemias. Distances were calculated as angles between vectors whose elements are the molecular-weight fractions (mol %) of the lipoprotein triglyceride component (top), or fatty acids (mol %) of the same lipoprotein triglycerides (bottom). Tips of vectors are projected on the same plane and relative distances were calculated.

CONCLUSIONS

(1) GLC carried out on a column with a low coating of stationary phase is able to analyse quantitatively high-molecular-weight fractions of triglycerides with very good reproducibility and sensitivity.

(2) Heterogeneous triglyceridemia in man is characterized by a relative elevation of high-molecular-weight fractions of plasma triglycerides.

(3) Molecular-weight distribution in comparison with the corresponding fatty acid profile of triglyceride components in the lipoprotein metabolic cascade was studied in a patient with familial type III dyslipoproteinemia and in a patient with secondary type IV hyperlipoproteinemia after a diet rich in very-long-chain fatty acids.

(4) In both patients molecular-weight profiling of triglycerides revealed a trend from randomness to non-randomness in the selection of fatty acid triplets for triglyceride molecules.

(5) For the detection of heterogeneous triglyceridemia and to follow the metabolism of high-molecular-weight fractions, intact lipid GLC profiling is a more informative method than fatty acid analysis.

REFERENCES

1 A. Kuksis, L. Marai and D.A. Gornall, J. Lipid Res., 8 (1967) 352.
2 S.L. Smith, M. Novotný, S.A. Moore and D.L. Felten, J. Chromatogr., 221 (1980) 19.
3 P. Mareš, E. Tvrzická and V. Tamchyna, J. Chromatogr., 146 (1978) 241.
4 P. Mareš, E. Tvrzická and J. Skořepa, J. Chromatogr., 164 (1979) 331.
5 J. Skořepa, P. Mareš, J. Rubličová and S. Vinogradov, J. Chromatogr., 162 (1979) 177.
6 J. Folch, I. Ascoli, M. Lees, J.A. Meath and F.N. LeBarron, J. Biol. Chem., 191 (1951) 833.