GAS CHROMATOGRAPHIC ANALYSIS OF THE FATTY ACID COMPOSITION OF THE PLASMA LIPIDS IN NORMAL AND DIABETIC SUBJECTS *

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Epidemiological, clinical-pathological, hormonal and nutritional studies during the last ten years indicate that disturbances of the lipid metabolism are important factors in the pathogenesis of atherosclerosis. Increased serum lipid level, induced by nutritional and pathological conditions, is correlated with an increased frequency of atherosclerosis. In diabetic acidosis, hyperlipemia (1–3) with a pronounced increase of free fatty acids (FFA) (4) is regularly found. After treatment with insulin and diet a normal serum lipid level usually occurs in patients with diabetes of short duration, although isolated increase of FFA often is found (5). Even in well controlled diabetic patients an elevation of the serum lipids is often seen when the diabetes has lasted for some years. Adlersberg and co-workers (6) reported that diabetic patients with vascular complications had significantly higher serum lipid levels than did cases with uncomplicated diabetes or normal controls. Lowy and Barach (7) found that this augmentation of the lipids preceded the symptoms of vascular complications.

In the present study a detailed analysis of the fatty acid composition of the different plasma lipid fractions in the postabsorbtive state was performed in seven diabetic patients and in eight healthy individuals used as controls.

MATERIALS AND METHODS

Six males with juvenile diabetes of varying duration, with diabetic vascular complications in different organs, were selected for this study. Later, one woman, who on several occasions had been found to have a high serum lipid level, i.e., 1,200 to 3,000 mg per 100 ml of plasma, was included, although her diabetes had started in adult life. Signs of diseases other than diabetes were not present. The basic diet was conventional Swedish food (excluding sugar, sweet fruits, and food with a high sugar content) with a high supply of protein and moderate restriction of bread and potatoes. Forty to 50 per cent of the total caloric intake was fat, most of it from milk, Swedish margarine (with a high content of coconut oil and rapeseed oil), pork and beef, with only a small part from fish and vegetables. No supplement of oils rich in polyunsaturated fatty acids was given. As a rule the fasting blood sugar level was below 0.200 g per 100 ml of blood and the daily output of sugar in the urine was between 10 and 30 g. Ketoacidosis was not present during the period of lipid analysis. The patients maintained a constant and normal body weight.

The ocular examination was performed by an ophthalmologist. The cardiovascular examinations included electrocardiography (leads I, II, III, IVR, CR_1, CR_3, CR_4 and CR_7) and X-ray examination of the heart, the thoracic and abdominal aortas, and the pelvic and leg arteries. In Case 3, the patient's history was compatible with coronary artery disease. The ECG was normal when the patient was resting but the exercise tolerance test was indicative of coronary insufficiency at 600 kilopond meters per minute. Signs of peripheral vascular insufficiency were not present in the patients, and oscillometric examinations of the extremities gave normal results.

When proteinuria developed during the disease it was taken as evidence of diabetic nephropathy, when repeated investigations failed to demonstrate other disease of the kidneys or the urinary tract. Advanced proteinuria and other signs of the nephrotic syndrome were not present.

At the time of lipid analysis all the patients lived their normal life and were capable of doing their normal work. The clinical data are summarized in Table I.

The eight healthy male human subjects studied were medical students and blood donors, 23 to 38 years of age. They lived on a standard Swedish diet with 30 to 40 per cent of the caloric intake as fat, most of it milk, Swedish margarine, pork and beef, with only a small part from fish and vegetables. One of them (B.J.) was a vegetarian. For several years he had endeavored to consume food containing fats with a very high content of polyunsaturated fatty acids.

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TABLE I

Clinical data relative to the diabetics

Patient	Age	Sex	Ht/Wt	Duration of diabetes	Insulin dosage	Plasma creatinine	Diabetic angiopathy
			cm/kg	*yrs*	*IU/day*	*mg/100 ml*	
J.O.E.	20	M	170/70	14	54	0.9	Retinopathy with capillary microaneurysm and retinal hemorrhages. Nephropathy with proteinuria.
A.B.	25	M	170/60	21	44	1.2	Advanced retinopathy with capillary microaneurysm, retinal hemorrhages, exudates and proliferating retinopathy. Angina pectoris. Vascular calcifications (X-ray) in leg vessels.
B.I.	29	M	163/65	28	64	0.9	Retinopathy with capillary microaneurysm.
H.O.S.*	20	M	165/62	18	64	0.8	Nephropathy with proteinuria.
L.O.S.*	20	M	165/58	12	68	0.7	Nephropathy with proteinuria.
Y.R.	42	M	173/69	32	64	1.6	Retinopathy with capillary microaneurysm, retinal hemorrhages and exudates.
G.B.	36	F	171/64	10	32	0.7	Retinopathy with capillary microaneurysm and retinal hemorrhages.

* Uniovular twins.

Experimental procedure

Blood for analysis was taken in the morning after the subjects had fasted for 10 to 12 hours. Blood sampling and preparation of the total lipid extracts were performed as described earlier (8).

Analytical methods. Total fat was determined by evaporation of an aliquot of the lipid extracts and weighing of the residue. Free and total cholesterol were determined according to the method of Zak and associates (9). FFA were determined according to the method of Svanborg and Svennerholm (8). The concentration was calculated as milligrams of oleic acid per 100 ml of plasma.

Glyceride-glycerol was determined as formaldehyde by the chromotropic acid color reaction according to Lambert and Neish (10), using the modification described by Carlson and Wadström (11). However, ethanol used in the saponification was found to give high and varying blank values. We therefore tried methanol, which was found to give low and constant blank values. From total lipid extracts the phospholipids were removed by chromatography on silicic acid (8). Recrystallized tripalmitin (B.D.H.) was used as standard and the triglyceride value was calculated as tripalmitin. Lipid phosphorus was determined according to Svanborg and Svennerholm (12).

Chromatographic separation of lipids. The plasma lipid classes were separated by the use of silicic acid chromatography as described by Hirsch and Ahrens (13). Silicic acid (Baker AR, lot no. 4680) was milled in a ball mill and screened. Particles passing a 120 DIN sieve were used. In order to remove extremely small particles the silicic acid was then suspended in methanol. After 5 minutes of settling, the methanol was decanted. This procedure was repeated once with methanol and twice with diethyl ether. The remaining silicic acid was dried in air and finally activated at 120° C for 24 hours. Sixty g of the silicic acid powder was suspended in ether and rapidly transferred to a glass column (Figure 1). The silicic acid column, which was about 18 cm in height, was washed with 100 ml of acetone-ethyl ether (1:1, vol/vol), followed by 100 ml of ether. Complete removal of the dehydrating solvents was then achieved by washing the column with 1 L of light petroleum (bp 60 to 80° C) for about 12 hours. A 60 g column was charged with 1 g of lipid. The mixture of lipids to be separated was dissolved in a small volume of light petroleum and pipetted on the column. Elution was carried out with solvent mixtures of increasing polarity, as is shown in Figure 2. The flow rate of the solvents was regulated to 2.5 ml per minute with the stopcock at the lower end of the column. Fractions of the eluate, 25 ml each, were collected. One-half of each fraction was used for gravimetric determination and colorimetric or titrimetric analysis (cholesterol, glycerol, phosphorus and FFA); the other half was used for gas chromatographic analysis of the fatty acids.

At the beginning of this investigation the possibility of using the same column in several separations was tested. However, the adsorption power of the silicic acid was found to decrease rapidly. Chromatography with standardized stepwise elution will not give reproducible results if the columns are used more than once.

Hydrolysis and esterification. The cholesterol esters and the triglycerides were hydrolyzed in 0.5 N ethanolic potassium hydroxide (10 ml for 100 mg lipid) by boiling under reflux for 2 to 3 hours. The reaction mixture was diluted with half the volume of water, and the unsaponifiable material was extracted with light petroleum. After

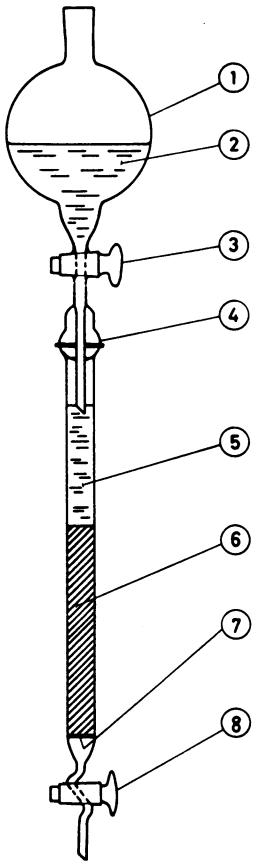

FIG. 1. GLASS APPARATUS USED FOR COLUMN CHROMATOGRAPHY WITH SILICIC ACID. 1 = two L separatory funnel; 2 and 5 = solvent; 3 and 8 = stopcocks; 4 = ball joint; 6 = silicic acid; 7 = coarsely sintered glass filter.

acidification the FFA were extracted from the ethanol-water phase with light petroleum. The solvent was evaporated and the fatty acids were converted into methyl esters by boiling with methanol containing 2 per cent (vol/vol) concentrated H_2SO_4 for 2 hours. (Too great a concentration of H_2SO_4 may lead to changes of the unsaturated acids.) The mixture was diluted with half the volume of water and the methyl esters were extracted with light petroleum. The light petroleum solution was washed with a sodium carbonate-bicarbonate buffer, pH 10, and water. The gas chromatographic analysis of a synthetic mixture of methyl stearate and methyl linolenate (stearate 61.5 per cent, linolenate 38.5 per cent) before and after hydrolysis and re-esterification (stearate 61.2 per cent, linolenate 38.8 per cent) showed that the treatment did not change the polyunsaturated fatty acids. In the first analyses the phospholipids were hydrolyzed and esterified by this method. In order to get a more complete conversion of the sphingomyelin fatty acids to methyl esters the phospholipids were boiled under reflux for 2 hours with methanol containing 5 per cent concentrated H_2SO_4. This procedure had been found to give complete methanolysis of brain cerebrosides. The relatively short reaction time was chosen in order to avoid changes in the polyunsaturated fatty acids. In one case both alkaline hydrolysis and methanolysis were used (see Table VI, "Blood plasma from 3 blood donors"). The principal difference is the higher amount of C_{24} acids split off from sphingomyelin by methanolysis than by alkaline hydrolysis. The proportion between linoleate and oleate is the same after methanolysis of the phospholipids as it is after alkaline hydrolysis, showing that the linoleate is not destroyed during methanolysis. This comparison can be made because the oleic and linoleic acids constitute only 1 per cent each of the sphingomyelin fatty acids.

After the completion of this investigation, however, it became obvious that the content of C_{24} acids was lower than could be expected from the sphingomyelin concentration in the phospholipids. In order to get more detailed information of the efficiency of the hydrolysis and methanolysis methods used, sphingomyelin prepared from human serum was subjected to alkaline hydrolysis and methanolysis. A mixture of 50 mg of sphingomyelin and 5 mg of heneicosanoic acid was boiled in 0.5 N ethanolic potassium hydroxide solution for 2 hours or in 5 per cent concentrated H_2SO_4 in methanol for 1, 2, 4 and 8 hours. The release of fatty acids from sphingomyelin was studied by gas chromatography using heneicosanoic acid as internal standard. During alkaline hydrolysis 20 to 25 per cent of the fatty acids were split off. Methanolysis for 1, 2, 4 and 8 hours split off 33, 50, 75 and 92 per cent of the fatty acids, respectively. The mean molecular weight of the fatty acids in human plasma sphingomyelin was found to be 326. Thus the fatty acids calculated as methyl esters should amount to 44 per cent of the sphingomyelin if the methanolysis is complete. The sphingomyelin constituted about 20 per cent of the phospholipids in serum. Since the sphingomyelin fatty acids amount to only about 10 per cent of the total phospholipid fatty acids, about 92 to 95 per cent of these acids are included in the present gas chromatographic analysis.

Gas chromatography. For gas chromatography (14, 15) Perkin-Elmer gas fractometers with thermistor thermal-conductivity cells as detectors were used. A Hewlett-Packard 425 A, direct current amplifier was used to amplify the detector voltage before recording. The outlet system of the apparatus had been modified to permit collection of fractions of substances with a high boiling point. For a good separation of substances with a high molecular weight it was necessary to increase the temperature of the injection block to about 30 to 40° C above the temperature of the column. The mixtures of the methyl esters of the fatty acids were analyzed on two stationary phases, viz., silicone (Dow-Corning high vacuum grease) and Reoplex 400 (Geigy) (16, 17). The silicone column packing consisted of 30 parts by weight of stationary phase to 100 parts of solid support (Sil-O-Cel, 50 to 75 mesh) which had been pretreated with acid and alkali and carefully washed with water. Before packing, the silicone column filling was heated at 330° C for 1 week in a slow stream of nitrogen (18). The silicone column (3 m) had about 2,800 theoretical plates for methyl stearate. Celite, 80 to 100 mesh, pretreated in

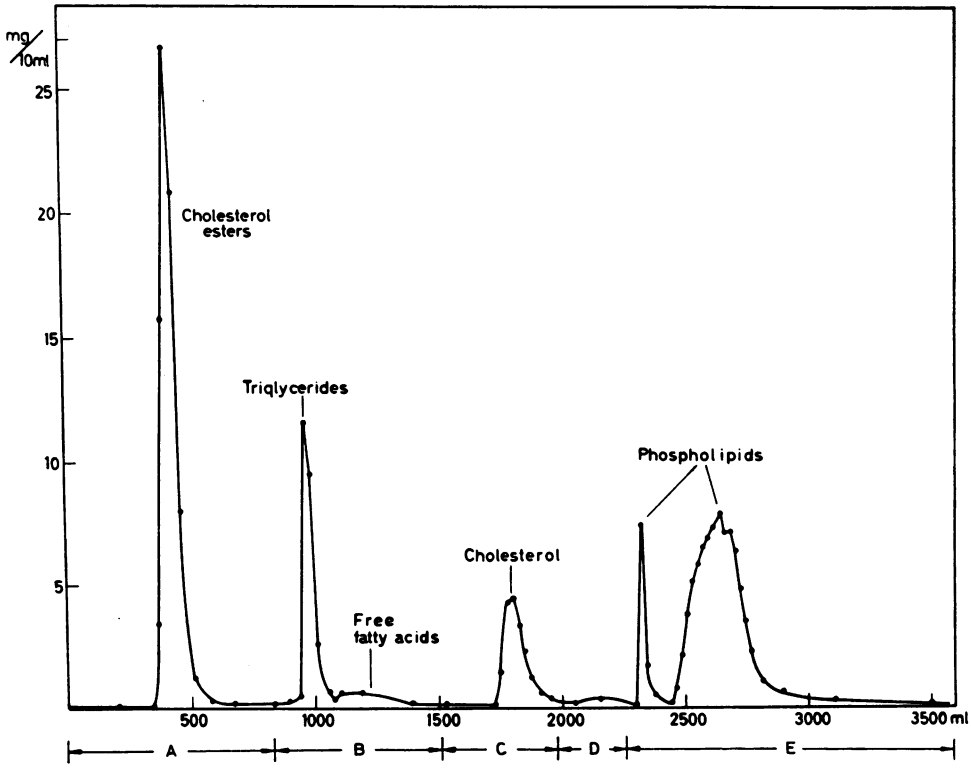

FIG. 2. THE SEPARATION OF THE PLASMA LIPID COMPONENTS BY SILICIC ACID CHROMATOGRAPHY. The following solvents were used: A, light petroleum, bp 60 to 85° C with 1 per cent diethyl ether; B, light petroleum with 8 per cent diethyl ether; C, light petroleum with 25 per cent diethyl ether; D, diethyl ether; E, methanol.

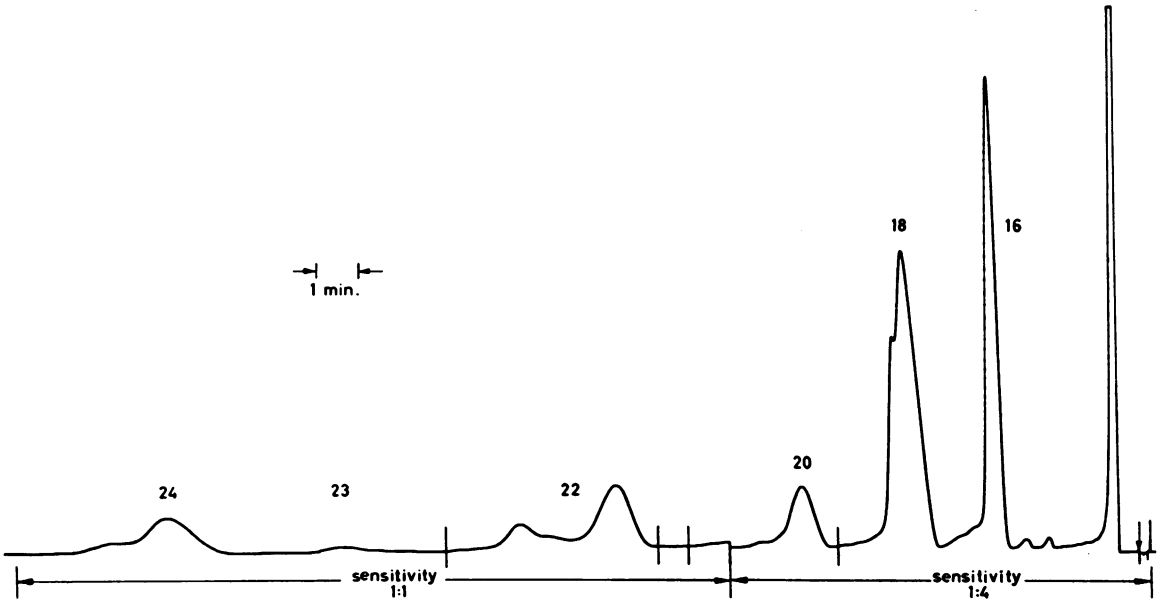

FIG. 3. GAS CHROMATOGRAM ON SILICONE OF THE FATTY ACID METHYL ESTERS OBTAINED FROM PLASMA PHOSPHOLIPIDS. The figures at the peaks denote the number of carbon atoms in the fatty acids. The methyl esters of the C_{22} acids are separated into two peaks, the first representing polyunsaturated acids, the second representing monounsaturated and saturated acids.

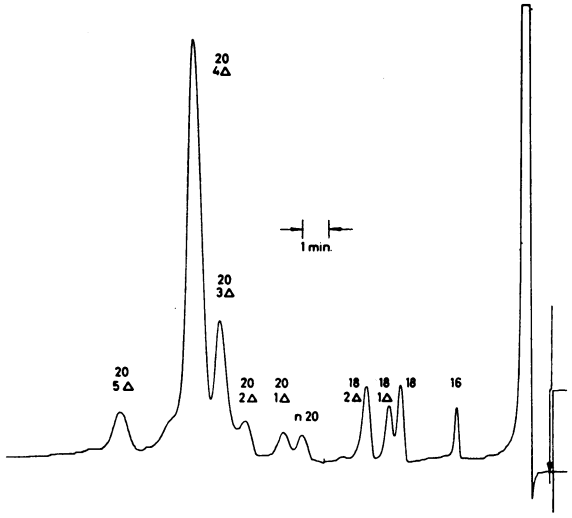

FIG. 4. GAS CHROMATOGRAM ON REOPLEX 400 OF THE METHYL ESTERS OF THE C_{20} FATTY ACIDS (COLLECTED BETWEEN THE SHORT VERTICAL LINES SHOWN IN FIGURE 3) FROM PHOSPHOLIPIDS. 1 △, 2 △, 3 △ and so forth, denote the number of double bonds. The esters of the C_{20} acids are contaminated with traces of those of C_{16} and C_{18} acids.

the same way as Sil-O-Cel was used as supporting medium in the Reoplex column. The same proportion by weight between stationary phase and solid support was used as that in the silicone packing. The Reoplex columns were preconditioned by heating at 240° C in a stream of helium (60 to 80 ml per minute) for at least 20 hours in order to minimize the bleeding and to shorten the retention times. The Reoplex column used (2 m) had 2,500 to 3,000 theoretical plates (for methyl stearate). On silicone the methyl esters separate principally according to the chain length, although the unsaturated esters have a shorter retention time than the saturated ones (Figure 3). Reoplex 400 gives a good separation of the unsaturated esters, the retention time increasing with the number of double bonds. Fractions containing acids of the same chain length were collected from the silicone column and injected into another apparatus containing a Reoplex column. The composition of the mixtures was calculated from the silicone curves and from the fractions analyzed on Reoplex (Figures 3 and 4). For identification of the peaks, logarithmic retention diagrams of the type shown in Figure 5 were used. As can be seen in this figure the methyl ester of eicosapentaenoic acid and docosenoic acid have the same retention time. The same is true for methyl docosapentaenoate and methyl tetracosenoate. These esters, however, separate on silicone. This separation can now be performed on polyesters other than Reoplex (17, 19).

Synthetic mixtures of methyl esters of saturated fatty acids from C_{16} to C_{22} were analyzed on silicone. There was good agreement between the observed values and the theoretical weight percentage composition (see Table II). Methyl esters of the fatty acids obtained from plasma cholesterol esters, triglycerides and phospholipids were

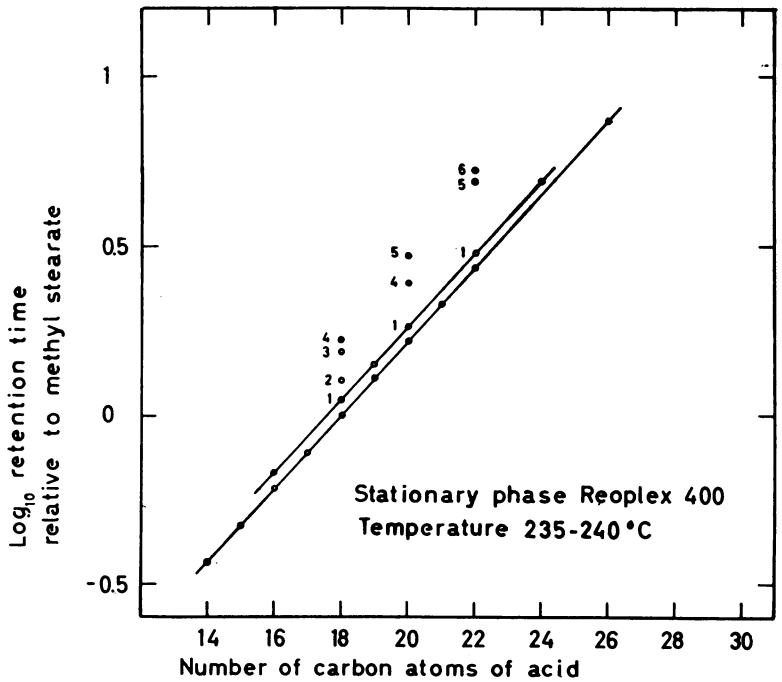

FIG. 5. RELATIONSHIPS BETWEEN THE NUMBER OF CARBON ATOMS OF THE FATTY ACIDS AND LOG_{10} RETENTION TIME RELATIVE TO METHYL STEARATE ON REOPLEX 400 AT 235 TO 240° C. The lower line represents esters of saturated straight chain fatty acids and the upper line represents monounsaturated esters. The figures denote the number of double bonds.

TABLE II

Gas chromatographic analysis on silicone of a synthetic mixture with approximately the same composition as the hydrogenated fatty acids from phospholipids (percentage composition)

	Observed values				Theoretical composition
	3 Repeated injections			Mean	
C_{16}	29.0	30.0	29.6	29.5	28.9
C_{18}	51.3	50.8	50.5	50.9	51.3
C_{20}	10.3	12.0	12.1	11.5	12.4
C_{22}	7.2	7.2	7.8	7.4	7.4

separated on silicone before and after hydrogenation. As can be seen in Table III, somewhat lower figures were found for the C_{20} and C_{22} fatty acids before hydrogenation, indicating a partial loss of the unsaturated acids. When the fatty acid composition was calculated from gas chromatographic analyses on silicone without hydrogenation, correction factors for the C_{20} and C_{22} acids amounting to 10 and 15 per cent of the found values, respectively, were used. These correction factors have been evaluated from many experiments. When mixtures of methyl stearate and methyl linoleate were analyzed on Reoplex columns, 98 per cent of the linoleate was recovered. We used a Reoplex column with short retention times to minimize the loss of polyunsaturated C_{20} and C_{22} fatty acids. To achieve a better separation, the C_{18} fatty acid methyl esters were analyzed at a lower temperature, 200 to 210° C.

TABLE III

Gas chromatographic analysis of plasma lipid fatty acids on a silicone column before and after hydrogenation (percentage composition)

	Cholesterol esters		Triglycerides		Phospholipids	
	Before	After	Before	After	Before	After
C_{12}			1.0	0.6		
C_{14}	2.1	1.5	3.9	3.4	1.0	1.1
C_{16}	15.2	15.4	28.9	28.6	31.9	30.2
C_{18}	75.7	75.2	60.3	60.3	46.8	46.6
C_{20}	6.5	6.9	3.4	3.8	11.7	13.0
C_{22}	0.6	1.0	2.6	3.3	6.1	7.2
C_{23}					0.2	0.4
C_{24}					2.3	2.1

RESULTS

The plasma lipid levels of the diabetic patients and the control subjects are given in Table IV. A typical elution curve from a separation on silicic acid is shown in Figure 2. A small peak of yellow material, probably carotene, appeared first. The colorimetric determinations showed that there were no glycerides in the cholesterol ester peak, neither was there any cholesterol in the triglycerides. In the cholesterol esters and in the triglyceride fraction the more saturated components were found to come first and the more unsaturated ones were concentrated in the tail of the peaks. Among

TABLE IV

Plasma lipid levels of normal control subjects and of patients with diabetes

Subjects	Age	Sex	Total lipids	Cholesterol		Phospholipids	Triglycerides	Free fatty acids
				Total	Free			
			mg/100 ml	*mg/100 ml*		*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
Normal cases:								
W.J.M.	38	M	640	204	58	170	121	17.0
L.H.	28	M	706	282	90	221	61	13.7
E.H.	23	M	591	204	62	209	58	16.8
Blood plasma from 3 blood donors	23	M	527	212	64	217	113	19.7
K.H.H.	23	M	474	217	72	168	29	28.0
Mean			588	224	69	197	76	19.0
Vegetarian:								
B.J.	26	M	424	128	43	150	76	19.0
Diabetics:								
J.O.E.	20	M	1,233	305	96	352	390	26.6
A.B.	25	M	1,120	438	142	343	98	48.0
B.J.	29	M	830	206	85	300	179	36.7
H.O.S.	20	M	520	141	62	212	62	11.8
L.O.S.	20	M	600	209	107	235	68	26.3
G.B.	36	F	1,145	275	113	355	318	43.7
Y.R.	42	M	942	387	109	323	99	32.4
Mean			913	280	102	303	173	32.2

TABLE V
Fatty acid composition of cholesterol esters

Subjects	C_{14}	C_{16}	C_{16} sat.	C_{16} monoene	C_{17}	C_{18} sat.	C_{18} monoene	C_{18} diene	C_{18} triene	C_{20} tetraene	C_{20} pentaene	C_{22} pentaene+ hexaene
Normal cases:												
W.J.M.	1.3	0.3	11.6	5.9	0.8	0.8	22.9	44.5	1.3	6.7	2.9	0.4
L.H.	1.5	0.5	10.4	4.5	1.0	1.1	22.7	48.6	1.6	5.3	1.4	0.8
E.H.	1.6	0.8	11.4	4.2	1.2	0.7	20.2	45.6	3.9	6.3	6.3	0.6
Blood plasma from 3 blood donors	1.3	0.7	10.6	4.3	0.9	0.9	21.3	47.9	1.4	6.3	2.1	0.5
K.H.H.	1.1	0.6	11.1	5.2	1.7	0.6	28.9	44.7		4.7	0.7	0.5
Mean	1.4	0.6	11.0	4.8	1.1	0.8	23.2	46.2	2.1	5.9	1.8	0.6
Vegetarian:												
B.J.	0.6	0.3	9.3	1.8	0.5	0.8	18.5	61.3		5.3	0.5	0.4
Diabetics:												
J.O.E.	1.2	0.3	10.4	5.5	1.3	0.8	26.0	45.9	3.1	4.4	0.6	
A.B.	0.9	0.4	11.4	3.2	1.3	0.7	18.0	52.6	1.9	7.5	1.2	0.7
B.J.	1.5	0.3	13.3	5.1	0.9	0.2	25.4	44.2	1.1	4.1	2.5	0.8
H.O.S.	0.9	0.5	9.8	6.5	1.4	1.0	24.6	43.7	2.8	4.0	1.6	
L.O.S.	1.2	0.4	11.8	2.7	0.9	1.0	24.4	50.6		5.4	0.8	0.4
G.B.	0.9	0.4	13.5	3.5	1.0	1.0	24.8	40.2	1.4	8.0	3.7	1.0
Y.R.												
Mean	1.1	0.4	11.7	4.4	1.1	0.8	23.9	46.2	2.1	5.6	1.7	0.7

the saturated esters, those with long chains were found to move faster than those with shorter chains.

Immediately after the triglycerides followed a small peak containing lipids with titratable acidity, the free fatty acids. The next peak, eluted by 25 per cent of diethyl ether in light petroleum, contained free cholesterol. Sometimes two small peaks giving glycerol reaction surrounded the cholesterol peak. The first one was probably diglyceride and the second monoglyceride, as described by Hirsch and Ahrens (13). Upon addition of methanol, the cephalins leave the column in the first peak. This peak also contains phosphorus free lipids, viz., cerebrosides and sulfatides, as could be demonstrated by paper chromatography (20). The second large peak consisted mainly of lecithin and sphingomyelin. The sphingomyelin and lysolecithin were found in the tail of the peak. This could be established by paper chromatography of the lipids and by analysis of the fatty acids for sphingomyelin.

The results of the plasma fatty acid analysis in the present investigation are shown in Tables V, VI, and VII. It can be seen that no definite difference between healthy individuals and diabetics on comparable diets was found. On the contrary, there was a remarkable similarity between the two groups. Striking differences were noted, however, in the levels of the various lipid fractions, as can be seen in Table IV. The diabetic group had a significant increase in the total lipid, cholesterol, phospholipid, triglyceride and FFA fractions.

One of the healthy individuals studied (B.J.) had been a vegetarian for several years. During the last six months he had included 40 to 60 g of corn oil daily in his diet. Among the different plasma lipid fractions, the highest increase of linoleic acid was found in the triglycerides. Linoleic acid constituted 39 per cent of the triglyceride fatty acids in this case as compared to 11 per cent in the other healthy individuals (Table VI). In the cholesterol esters the increase was from 46 to 61 per cent and in the phospholipids from 21 to 29 per cent. Our results thus confirm the findings of Ahrens and co-workers (21) that the phospholipids appear to have the most constant fatty acid pattern of any serum lipid group. The triglycerides and cholesterol esters more strongly reflect the changes in dietary fats.

The results of the fatty acid analysis in the different plasma lipid fractions have been used for studying the validity of the conventionally used factors for calculation of the amount of cholesterol esters from cholesterol determination, triglycerides

TABLE VI

Fatty acid composition of triglycerides

Subjects	C_{14}	C_{15}	C_{16} sat.	C_{16} monoene	C_{17}	C_{18} sat.	C_{18} monoene	C_{18} diene	C_{20} monoene	C_{20} tetraene	C_{20} pentaene	C_{22} pentaene	C_{22} hexaene
Normal cases:													
W.J.M.	2.3	0.4	30.0	4.6	1.2	4.4	40.3	11.4	1.0	1.0	0.2	0.5	1.0
L.H.	3.5	1.1	20.4	17.1	2.0	4.7	40.0	12.8	1.7	1.0	0.7	0.7	1.7
E.H.	3.1	1.0	24.8	8.2	2.2	3.1	39.3	12.0	0.9	0.7	0.8		
Blood plasma from 3 blood donors	4.2	1.4	25.0	5.3	2.0	4.7	38.2	9.1	1.9	0.8		0.5	0.7
K.H.H.	2.1	0.7	24.3	5.9	1.7	3.1	49.0	9.3	1.6	0.5		←1.0→	
Mean	3.0	0.9	24.9	6.2	1.8	4.0	41.4	10.9	1.4	0.8	0.6	0.6	1.1
Vegetarian:													
B.J.	1.1	0.4	15.2	3.3	0.5	2.0	31.9	39.0	0.6	1.6		0.2	0.1
Diabetics:													
J.O.E.	2.8	1.0	27.7	5.9	1.8	5.1	42.9	8.1	1.0	0.6	0.1	0.4	0.4
A.B.	2.1	0.4	21.5	5.0	4.6	5.8	39.6	13.9	1.2	1.1		0.7	1.2
B.J.	2.3	0.6	26.2	6.9	1.9	4.0	38.2	10.3	1.8	0.9	0.5	0.5	1.6
H.O.S.	3.3	1.3	27.4	6.1	2.7	4.3	40.7	7.0	←	2.6	→	←1.9→	
L.O.S.	1.9	0.5	29.4	3.9	1.0	5.1	41.8	11.5	1.5	0.9	0.1	0.5	0.7
G.B.	2.1	1.0	26.6	6.2	1.9	4.7	33.1	14.7	1.1	1.4	0.6	1.0	1.9
Y.R.	2.5	0.6	29.3	2.5	1.2	4.7	38.8	12.1	←	3.5	→	←3.7→	
Mean	2.4	0.8	26.9	5.2	2.2	4.8	39.3	11.1	1.3	1.0	0.3	0.6	1.2

TABLE VII

Fatty acid composition of phospholipids

Subjects	C_{14}	C_{15}	C_{16} sat. + monoene	C_{17}	C_{18} sat.	C_{18} monoene	C_{18} diene	C_{20} triene	C_{20} tetraene	C_{20} pentaene	C_{22} pentaene	C_{22} hexaene	C_{24} monoene	
Normal cases:														
W.J.M.	0.5	0.8	29.6	2.1	11.2	13.7	18.0	2.7	10.6	2.6	1.4	5.3		A*
L.H.	0.6	1.0	26.0	2.2	11.8	15.9	20.7	1.8	9.5	1.7	1.1	6.2		A
E.H.	0.5	0.6	24.7	2.0	13.2	14.7	21.2	3.1	9.3	2.0	1.6	4.1		A
Blood plasma			28.1	1.1	9.6	12.5	24.3	2.0	8.7	2.2	0.5	4.8	1.4	A
from 3 blood donors	0.6	0.4	27.2	0.8	11.5	11.9	22.5	1.9	7.5	1.9	0.7	3.4	4.1	M†
K.H.H.	0.5	0.4	30.9	1.0	11.7	15.3	19.8	2.6	7.0	0.8	4.0		2.9	M
Mean	0.5	0.6	27.7	1.6	11.9	14.3	20.4	2.4	8.8	1.8	1.2	4.8		
Vegetarian:														
B.J.	0.3	0.5	22.8	0.8	15.5	10.0	29.1	3.2	12.4		0.2	2.0	0.9	A
Diabetics:														
J.O.E.	0.5	0.4	30.0	1.5	10.8	19.6	20.1	2.8	8.1	0.8	1.2	2.0	0.4	A
A.B.	0.3	0.7	28.6	1.2	11.1	13.7	23.0	1.4	10.7	0.8	0.8	4.9	0.7	A
B.J.	0.4	0.4	28.7	0.5	12.4	13.8	18.0	1.8	6.4	1.8	1.2	5.9	3.1	M
H.O.S.	0.8	0.6	30.9	1.1	10.8	15.9	20.3	2.7	6.2	2.1	2.2	1.8	2.5	M
L.O.S.	0.3	0.3	28.7	0.8	12.8	13.1	24.2	2.5	7.8	0.9	1.0	2.3	2.0	M
G.B.	0.4	0.3	29.9	1.2	11.4	12.8	16.3	1.8	10.3	2.3	1.1	6.0	2.5	M
Y.R.	0.4	0.5	28.5	1.0	14.8	13.0	18.3	1.9	8.5	0.9	0.4	2.9	3.0	M
Mean	0.4	0.5	29.5	1.1	12.0	14.6	20.0	2.1	8.3	1.4	1.1	3.7	2.6	

* A = alkaline hydrolysis.
† M = methanolysis.

from glycerol, and phospholipids from phosphorus determination. The mean molecular weight of the fatty acids of the cholesterol esters was found to be 276 and of the triglycerides 272. The factor for calculating the phospholipids from phosphorus analysis was checked by comparison with the gravimetric determinations of the phospholipids after chromatographic separation. This factor was found to be 25.

DISCUSSION

In order to avoid cases with senile atherosclerotic vascular disease, juvenile diabetics and one middle-aged patient with adult diabetes were selected for this study. Three of the patients studied had early signs of vascular complications in different organs and four had signs of more advanced complications. When the lipid analyses were performed rapid progression of the vascular complications was present in most of the patients. Therefore, it seems reasonable to assume that changes in the fatty acid composition of the plasma lipids should have been demonstrable in these patients if such changes really were of pathogenic significance in diabetic vascular disease.

The knowledge of the disturbances of the lipid metabolism in diabetes is only fragmentary. It is clear from many lines of research that the synthesis of both cholesterol and fatty acids is greatly influenced by the metabolism of glucose (22, 23). Decreased synthesis both of fatty acids and cholesterol is reported in diabetes and the cause of this lipogenetic defect was recently discussed by Siperstein and Fagan (23). The finding that the FFA in plasma often are abnormally high, even in well controlled diabetic patients (5), indicates an abnormally high mobilization of fatty acids from fat depots in diabetes. As has been mentioned earlier, other plasma lipid fractions also, especially the triglycerides, are often found to be abnormally high in diabetics. According to our experiences, the postabsorptive plasma lipid level shows greater variability from day to day in diabetics than it does in healthy individuals.

Atherosclerosis is a metabolic disease in which altered lipid metabolism seems to play an important role. Numerous investigators have studied the levels of the plasma lipids in individuals with atherosclerosis during middle age and found an elevated plasma lipid level to be a cardinal sign of the disease. The disturbances of the lipid metabolism in diabetes and the tendency to hyperlipemia have been proposed to be the pathogenic factors responsible for the high frequency of atherosclerosis among diabetics.

Dietary fats rich in polyunsaturated fatty acids have been found to decrease the plasma lipid level and to have a prophylactic and therapeutic effect on atherosclerosis in experimental animals (24). Some investigators have therefore proposed that there might be a deficiency of polyunsaturated fatty acids in patients with atherosclerosis. However, in a study of the plasma fatty acid composition of the phospholipids and of the acetone-soluble fraction of plasma lipids, James, Lovelock, Webb and Trotter (25) failed to demonstrate any difference between healthy individuals and patients with ischemic heart disease. In the present investigation of diabetics with vascular complications a more detailed analysis of the fatty acids in the different plasma lipid fractions has been performed. The separate analysis of the triglyceride fraction seems to be of special interest, since this fraction is often elevated in the plasma of diabetics when cholesterol esters and phospholipids lie within normal limits.

The present investigation has demonstrated that diabetics and healthy individuals of the same age, on comparable diets, have the same plasma fatty acid composition. These observations contradict the hypothesis that a deficiency of polyunsaturated fatty acids is an essential factor in the pathogenesis of the vascular complications in diabetes. It is more probable that the increased levels of the different plasma lipid fractions play an important role. The largest percentage increase was found for the triglycerides, which is in accordance with earlier findings (6). The dietary management of diabetes must be directed toward a normalization not only of the blood sugar level but also of the blood lipid level.

SUMMARY

The fatty acid composition of cholesterol esters, triglycerides and phospholipids was studied by gas chromatographic analysis in seven diabetics with vascular complications and in seven healthy individuals of the same age on a similar diet. The fatty acid composition of the different plasma lipids was found to be remarkably similar in the two groups. The only pathological plasma lipid

finding demonstrable in the diabetics was an abnormally high plasma lipid level in some cases.

During chromatogarphy on silicic acid the more saturated cholesterol esters and triglycerides were found to move faster than the more unsaturated ones.

The accuracy of the conventionally used factor of 25 for calculating phospholipids from phosphorus determinations was substantiated. In the average subjects the mean molecular weights of the cholesterol ester fatty acids and triglyceride fatty acids were found to be 276 and 272, respectively.

In an additional study of the plasma lipids of a healthy vegetarian consuming 40 to 60 g of corn oil per day, the most marked increase of linoleic acid was found in the triglycerides along with a smaller increase in the cholesterol esters. The phospholipids were found to have the most constant fatty acid pattern.

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