Serum Phospholipids in Diabetic Patients with Late Manifestations

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Summary. Serum phospholipid constituents in a selected group of hyperlipaemic diabetic patients with late manifestations have been investigated. In these patients the concentrations of serum lecithin as well as the cephalin fraction were increased. The absolute and relative increase of the cephalin fraction amounted to 3–4 times. It is indicated that the disturbances in serum phospholipids are due to insufficient metabolic control in these patients. The possible significance of the increase of serum cephalin for the occurrence of vascular complications is discussed.

Phospholipides sériques chez des patients diabétiques ayant des manifestations tardives

Résumé. Les auteurs ont étudié les phospholipides du sérum dans un groupe sélectionné de patients diabétiques hyperlipémiques ayant des manifestations tardives. Les concentrations de lécithine sérique ainsi que la fraction céphaline étaient accrues chez ces patients. L'augmentation absolue et relative de la fraction céphaline atteignait

des valeurs de 3 à 4 fois. La signification possible de cette augmentation pour les complications vasculaires est discutée.

Serumphospholipide beim spät-manifesten Diabetes

Zusammenfassung. Die Bestandteile der Serumphospholipide wurden in einer ausgewählten Gruppe von hyperlipämischen Patienten mit spät-manifestem Diabetes untersucht. Bei diesen Patienten waren sowohl die Konzentrationen des Serumlecithin als auch der Kephalinfraktion erhöht. Der absolute und relative Anstieg der Kephalinfraktion betrug das 3–4-fache. Es wird angenommen, daß die Störungen im Stoffwechsel der Serumphospholipide auf die ungenügende Stoffwechselkontrolle bei diesen Patienten zurückzuführen ist. Die mögliche Bedeutung des Anstiegs des Serumkephalin wird in Hinblick auf das Eintreten von Gefäßkomplikationen diskutiert.

Key-words: Phospholipids, cephalin, insulin, diabetes, serum.

The relationship between an elevation of phospholipids in plasma and the incidence of vascular diseases has captured attention in recent years. Special emphasis has been given to the increase in the cephalin fraction in patients with coronary heart disease and hyperlipaemia [15].

Very little is yet known about the distribution and behaviour of the different phospholipids in the blood of diabetic patients with vascular complications. The aim of the present work, therefore, has been to investigate the total and the individual phospholipids in a selected group of diabetic patients with late manifestations. Since evidence has been obtained that the surface charge of the phospholipids is a most important factor in the process of blood coagulation [10], special emphasis has been given to the determination, not only of the total concentrations of the individual phospholipids, but to the relative distribution, especially of lecithin and cephalins, as separated and quantitated by thin layer chromatography. The patients were selected according to investigations on lipoprotein pattern by paper electrophoresis, and determinations of the concentrations of total cholesterol and triglycerides.

Materials and Methods

The group of diabetic patients described in the present communication were all under observation in

the University Clinic, Rikshospitalet, Department of Medicine B, Oslo. They were selected being all of the juvenile type treated with insulin. A case report is given for each patient.

Case I. K.B. f. Age 58 y. Age at the onset of diabetes: 25 y. Duration of illness: 33 y. Weight: 90 kg. Height: 162 cm. The diabetes was badly regulated, and insulin 'feelings' frequently occurred. A diabetic retinopathy with impaired vision in both eyes was present. The blood pressure was 260/140, being treated with methyl-dopa. No cardiac murmurs were heard and ictus cordis was not laterally displaced. Proteinuria and tibial oedema were found. However, the concentration of blood urea and creatinine were within normal limits. In addition, a slight diabetic neuropathy with lowered tendon reflexes and sensibility was demonstrated. The blood serum showed no opalescence, and the lipoprotein lipase activity was considered normal.

Case II. R.F. U. f. Age 44 y. Age at the onset of diabetes: 12 y. Duration of illness: 32 y. Weight: 61 kg. Height: 169 cm.

A positive family history of diabetes was established. The patient was amaurotic in both eyes because of a proliferative diabetic retinopathy. She was only slightly hypertensive (BP: 155/100). A rough systolic murmur all over the praecordium with an accentuated A₂ was heard. She had a necrotic ulcer on the right leg. Oedema in the lower limbs was present. No diabetic

nephropathy was found. Serum albumin was low, with a concurrent increase in A₂-globulin (1.1 g/100 ml). Tendon reflexes were missing, indicating diabetic neuropathy. The blood serum showed no turbidity and the lipoprotein lipase activity was normal.

Case III. R.H. f. Age 42 y. Age at the onset of diabetes: 16 y. Duration of illness: 26 y. Weight: 45 kg. Height: 158.5 cm.

The regulation of the diabetes was difficult, and insulin 'feelings' frequently occurred. A diabetic retinopathy was diagnosed in 1959, and from 1963 amaurosis in the left eye was present, then progressive blindness in the right eye. No hypertension was found, for a period the patient was even orthostatic hypotensive (BP: 100/65). No cardiac murmurs were heard. A progressive nephropathy with reduced renal function was present (proteinuria, urea 149 mg/100 ml, creatinine 5.1 mg/100 ml) and slight hypoalbuminaemia and a slight reduction in the level of serum potassium. In addition, the patient had neuropathy at an early stage of development with reduction in the sense of vibration. The serum was without turbidity, and the lipoprotein lipase activity was normal.

Case IV. T.B.K. f. Age: 29 y. Age at the onset of diabetes: 13 y. Duration of illness: 16y. Weight: 60 kg. Height: 173 cm.

A diabetic retinopathy was present, surgically treated with hypophysectomy resulting in visus 6/8.5 in the left eye, enabling her to read. Obesity or hypertension were not present and there were no signs of coronary heart disease or peripheral vascular disease. Diabetic nephropathy or neuropathy could not be demonstrated, no proteinuria, normal serum creatinine and urea, all the reflexes intact. The serum was clear with normal lipoprotein lipase activity.

Case V. L.N.m. Age: 54 y. Age at the onset of diabetes: 30 y. Duration of illness: 24 y. Weight 60 kg. Height: 172 cm.

The diabetes was well regulated. The patient was not excessively obese, arterial hypertension was found (BP: 225/100); atherosclerotic degenerations, especially in the radial arteries were demonstrated. From May 1966 cardiac failure was successfully treated with digitalis. A rough, systolic cardiac murmur was heard, crepitations basally in both lungs, and hepatosplenomegaly were found. The renal function was normal, moderate tibial oedema was present, but no proteinuria. The patient had a moderate, progressive neuropathy with cerebral and peripheral manifestations. His serum was not turbid, and the lipoprotein lipase activity was considered normal.

Case VI. K.O. f. Age: 24 y. Age at the onset of diabetes: 14 y. Duration of illness: 10 y. Weight: 62 kg. Height: 169 cm.

The diabetes was badly regulated. The father, brother and a paternal aunt were diabetics. The patient was slightly hypertensive. An open foramen ovale was surgically restituted in 1968. No diabetic retinopathy was present. The renal function was normal with no

proteinuria or oedema. Serum creatinine and urea values were within normal limits. No diabetic neuropathy had so far been demonstrated. No opalescence of the serum was seen, and the lipoprotein lipase was normal.

In Table 1 the patients are presented individually with special emphasis on the duration of illness and the state of complications. The metabolic control of the patients was based upon diurnal blood sugar levels, excretion of urinary sugar, changes in insulin requirement and general clinical evaluation during some weeks preceeding the examination of serum lipids. A satisfactory metabolic control had not been achieved in these patients except Case V (L.N.). It is seen that retinopathy was most common among late manifestations in this group of patients.

Blood sampling. The blood samples were collected after 14 h of fasting without use of anticoagulant. The samples were allowed to coagulate, chilled and centrifuged at 4°C for 10 min at 2000 r.p.m. After immediate separation the sera were kept in the cold and protected from light to avoid autoxidation, which can give an unspecific increase in the lysophospholipid compounds. The sera were investigated on the day of sampling.

Lipid extraction. A serum lipid extract was prepared according to Svennerholm¹. Conical tubes with glass stoppers were used for the extraction procedure. To 6 ml chloroform-methanol mixture (1:1, v/v) was added 0.5 ml serum dropwise with vigorous shaking on a Vortex mixer. The samples were left at room temperature for 30 min for complete precipitation of the proteins. The precipitate was packed by centrifugation at 2500 r.p.m. for 10 min. The supernatant fluid was transferred to a second conical tube, the precipitate was washed with 2 ml chloroform-methanol (1:1, v/v) and the washing added to the supernatant fluid. Thereupon, 4 ml chloroform and 2 ml acid-salt solution (0.1% NaCl + 0.002 N H₂SO₄) were added, and a vigorous shaking was performed for the phase separation. After centrifugation for 10 min at 2500 r.p.m., the upper aqueous-methanol phase was removed with a Pasteur pipette and suction. The walls of the tube and the surface of the chloroform layer were washed twice by the careful addition of 1 ml washing reagent (prepared by mixing 15 ml chloroform, 260 ml methanol and 250 ml 0.1% NaCl), which was removed by suction. The lower chloroform phase was transferred to a 10 ml cylinder, and adjusted to the mark with methanol. This serum-lipid extract was kept at -18° in the dark before use. Aliquots were taken for evaporation to dryness under nitrogen in a waterbath at 40 - 50°C, protected from light.

Thin layer chromatography. Chromatography was performed on glass plates (200 × 200 mm), covered with silica gel G (E. Merck AG, Darmstadt, Germany). A slurry was made by mixing 40 g of silica gel G with 90 ml water for neutral plates or 90 ml of 0.005 M Na₂

¹ personal communication

CO₂-solution for basic plates. The slurry was transferred to the applicator and applied on the plates as a layer of 0.5 mm thickness. The plates were allowed to dry at room temperature for 1–2 h, and were stored in a cabinet without drying agent. Before the experiment the plates were activated at 110°C for 1 h.

The dried lipid extracts were redissolved in an appropriate volume 25–50 µl chloroform, and applied along a line 1.5 cm long with standardized micropipettes. Additional 25 µl chloroform was used to wash the tubes and this volume was applied on the same line. Routinely, 5 ml of the serum-lipid extract was evaporated for each application for the determination of lysolecithin², sphingomyelin and cephalin. For convenience, the quantitative determination of the lecithin spot was performed by evaporating 1 ml serum-lipid extract and was run on separate plates. Triplicates were run from each serum. Up to three serum-lipid extracts were

follows: After moistening with a drop of water the spot could be scraped off and transferred to a Pyrex tube for ashing. A complete transfer was obtained by the use of a fitted spatula. Blanks from powder were cut from parts of the gel where no lipids had been applied. No phosphorus was found in these spots. The determination of total lipid P in serum was done on 1 ml serum-lipid extract (corresponding to 0.05 ml serum).

Phosphorus determination. The quantitative determination of the phospholipid samples was done essentially according to the method of Bartlett [2]. The digestion was performed in calibrated Pyrex tubes with 0.5 ml 10 N H₂SO₄. The ashing procedure was performed in a sand-bath at 180° to 230°C for 1½ h. After cooling, three drops of H₂O₂ were added, and the tubes reheated for 15 minutes. Thereupon the walls were washed with 0.4 ml water and the solution boiled for 15 min. Then 3.0 ml water, 0.4 ml 2.5 per cent ammoni-

Table 1. *Characterization of the diabetic patients*

Patient	K.B.	R.F.U.	R.H.	T.B.K.	L.N.	K.O.
Sex	f	f	f	f	m	f
Age	59	44	42	29	55	25
Type	juvenile	juvenile	juvenile	juvenile	juvenile	juvenile
Duration of illness	33 y	32 y	27 y	16 y	20 y	11 y
Need of insulin (units)	60+36	16+16	36	12 + thyroxine + cortisone	28+12	32+28
Metabolic control	poor	poor	poor	poor	Good	poor
Retinopathy	+	+	+	+	+	—
Nephropathy	+	+	+	—	—	—
Neuropathy	+	—	—	—	—	—
Hypertension	—	+	+	—	+	+

prepared from each serum sample and pooled before the aliquots were taken for evaporation. Reference compounds³, each corresponding to 3–4 µg phosphorus were dissolved in chloroform and applied on the plate, in addition to the three lines with extracts. One dimensional chromatograms were routinely developed with chloroform-methanol-water (65:25:4 by volume). Additional runs were done according to Skipski *et al.* [21] in chloroform-methanol-glacial acetic acid-water (25:15:4:2). The running time was 1–1½ h (12 cm from the application spot). The plates were air dried at room temperature for 30 min. The spots were detected with iodine vapour and encircled with a needle. According to the amount applied no spot exceeded 1.5 × 1.5 cm. The removal of the spots was performed as

2 Abbreviations used: LE: lecithin, LLe: lysolecithin, Sp: sphingomyelin, PE: phosphatidylethanolamine, LPE: lysophosphatidylethanolamine, PS: phosphatidylserine, Pl: phosphatidylinositol, Ce: cephalin fraction.

3 Reference compounds: Highly purified lecithin, lysolecithin, phosphatidylethanolamine and lysophosphatidylethanolamine were available, thanks to the courtesy of Dr. E. Gjone, Rikspospitalet, Oslo. Sphingomyelin (bovine brain), Sigma 56B-0320. Phosphatidylinositol (bovine brain, Folch fraction I), Koch-Light 25388. Phosphatidylserine (bovine brain, Folch fraction III) Koch-Light 38406.

um molybdate and 0.2 ml Fiske-SubbaRow's reagent were added and the sample diluted to the 5 ml mark. The reagents were mixed and heated for 7 min in a boiling water bath. After colour development the silica gel was removed by centrifugation at 2500 r.p.m. for 10 min. Optical density was read at 830 mµ. An OD reading of 0.12 was given by 1 µg P (KH₂PO₄). Recovery of lecithin standard from the plate was 99 per cent, of the cephalin fraction (phosphatidylethanolamine + phosphatidylserine) was 90 per cent. The average recovery for the serum lipid extracts amounted to 91.5 per cent, which is in good agreement with the recoveries given by Gjone and Orning [7].

The serum triglycerides were determined according to Laurell [12]. The serum cholesterol was determined by the method of Carr and Dreker [4]. Lipoprotein paper electrophoresis in albumin-containing buffer was performed according to Lees and Hatch [13].

Comments on the method. Determination of the individual phospholipids in serum under normal and pathological conditions requires careful analytical precautions to avoid nonspecific variations in the concentration of the compounds.

Blood sampling. As discussed by Vikrot [22], phospholipids may be liberated from the blood cells during

coagulation, especially from the platelets, and bound to the clot. In the present work a substantial coagulation was present before chilling and no haemolysis occurred. No counting of platelets was performed. In heparinized plasma Gjone *et al.* [6] found no difference in the concentration of the individual lipids compared with the values found in serum [7]. By counting the platelets [7] in heparinized plasma and citrated plasma the number of platelets did not influence the concentration of lysolecithin, lecithin and sphingomyelin, and only a slight increase in the cephalin fraction could be observed. The standardized procedure for blood sampling used in the present work, therefore, excludes the possibility that differences in cephalin fraction could depend upon the technique of blood sampling.

Extraction procedure. In the present work the precipitation of the serum proteins was done with the chloroform-methanol mixture 1:1 (v/v) and the phase separation was performed with a 2:1 (v/v) mixture, as commonly used. It is of interest that the values obtained for total lipid P and for the individual lipids in the control group (Table 1) show good correspondence with the values given by Gjone and Orning [7], in spite of different volumes and different salt concentration used.

Thin layer chromatography. With the routine procedure described, four distinct spots could be identified on the chromatograms, namely lysolecithin ($R_f: 0.15 \pm 0.02$), sphingomyelin ($R_f: 0.27 \pm 0.04$), lecithin ($R_f: 0.47 \pm 0.03$) and cephalin ($R_f: 0.87 \pm 0.03$).

As judged from reference compounds, no separation was obtained for the phosphatidylethanolamine and phosphatidylserine in the system. In separate experiments using the systems of Skipski *et al.* [21] complete separation of these compounds was not obtained on either neutral or basic plates. The apparent single spot with the R_f -value of 0.87 is referred to as the cephalin fraction. The spot was distinct and showed a positive ninhydrin reaction. The three other spots showed a positive Dragendorff reaction [24] due to the presence of choline. These spots were all distinct without 'tailing'. No tendency for the sphingomyelin to separate into two spots was observed. The lysophosphatidylethanolamine ($R_f: 0.45$), if present in the extract, would be included in the lecithin spot.

Results

Individual serum phospholipids in non-diabetics. The concentration of phospholipids in sera from the control group of non-diabetics is presented in Table 2. The material consists of young medical students, 20–23 years old. Included in Table 2 are the values obtained from young controls, aged 20–24 years, reported by Vikrot [22] and the controls given by Gjone and Orning [7], aged 20–40 years. These values were obtained by separation of the individual phospholipids by the method of thin layer chromatography as used in the present work. Nelson and Freeman [14] have presented a con-

Table 2. Serum phospholipids in normal individuals. Values obtained in the present study compared with values from the literature

Material	TPL mg per 100 ml	LLe mg per 100 ml	%	Sp mg per 100 ml	%	Le mg per 100 ml	%	Ce mg per 100 ml	%
Non-diabetics (present study) Age 20–23 years Number = 8	Mean S.E.M. Range 188 ± 4 175–202	10.0 ± 1.0 5.8–15.8	5.3 ± 0.5 3.3–7.9	34.4 ± 1.8 27.3–40.2	18.3 ± 1.1 13.7–23.0	136.0 ± 3.8 120.0–150.0	72.3 ± 1.0 68.0–76.5	7.7 ± 1.1 4.2–14.5	4.1 ± 0.6 2.2–7.4
Controls (Vikrot (22)) Age 20–24 years Number = 9	Mean 219	5.9	21.2				69.9		3.0
Controls (Gjone and Orning (7)) Age 20–40 years Number = 15	Mean Range 193 151–213	5.5 3.7–9.3	17.3 12.6–21.8				73.1 68.6–77.5		4.1 2.9–5.6
Blood donors (Nelson and Freeman (14)) Age 34–55 years Number series A: 10 series B: 5	Mean A B						74.4 73.3		6.1 6.5

trol group of higher age, 34–55 years, with a per cent distribution of individual phospholipids not different from the range found at younger age. The control values of the individual phospholipids in the present work correspond very well with the values given by the authors mentioned.

Individual serum phospholipids in diabetics. The level of total phospholipids and the distribution of individual phospholipid components in sera from the diabetic patients are shown in Table 3. Five of these patients had an elevated level of total phospholipids, the highest value being 321 mg/100 ml (range of control group 175–202 mg/100 ml). This increase of total

phospholipids in the diabetics was somewhat different from the control group. The marked increase in the cephalin fraction is reflected in a higher percentage of this group compared with the controls (mean values of diabetics 12 per cent against 4 per cent in normals). Correspondingly, the percentage distribution of lecithin is somewhat decreased (mean values in diabetics 60 per cent against 72 per cent in normals).

Determination of other lipid constituents in sera of the diabetics. The values of serum cholesterol, serum triglycerides as well as the lipoprotein pattern in the diabetic patients are also presented (Table 4). Four of the patients revealed some abnormalities. These patients

Table 3. Serum phospholipids in diabetic patients

Patient	TPL mg/100 ml	LLe mg/100 ml	%	Sp mg/100 ml	%	Le mg/100 ml	%	Ce mg/100 ml	%
K.B.	272	28.0	10.0	47.6	17.0	151	63.3	55.5	19.7
R.F.U.	293	24.4	8.3	36.0	12.4	180	61.5	52.2	17.8
R.H.	266	6.3	2.5	33.4	12.5	206	77.5	20.0	7.5
T.B.K.	321	45.0	14.0	56.6	17.6	182	59.6	37.8	11.8
L.N.	258	29.8	11.5	56.8	22.0	160	62.0	11.7	4.5
K.O.	201	15.2	7.6	56.8	28.2	101	49.8	28.8	14.4
Mean	270	24.8	9.1	47.8	17.7	164.0	60.6	34.4	12.6
S.E.M.	±17	±5.1	±2.6	±4.4	±2.4	±14.7	±3.9	±7.1	±2.4
Range	201–321	6.3–45.0	2.5–14.0	33.4–56.8	12.4–28.2	101–206	49.8–77.5	11.7–55.5	4.5–19.7
<i>p</i> ^a :	>0.001	0.01	0.025	<0.10	<0.10	>0.001	0.005	0.001	>0.005

^a Test of significance diabetics versus controls

Table 4. Lipid constituents in sera of diabetic patients

Patient	Lipoprotein electrophoresis	Serum- cholesterol. mg per 100 ml	Serum- triglycerides. mg per 100 ml
K.B.	hyper-beta-lipoproteinaemia	440	120
R.F.U.	Elevation of the pre-beta-LP fraction	345	115
R.H.	Hyper-pre-beta-lipoproteinaemia	330	216
T.B.K.	Hyper-beta-lipoproteinaemia	400	128
L.N.	normal	260	104
K.O.	normal	260	83

phospholipids was mainly due to an increase in the lecithin and in the cephalin fraction. The lecithin fraction was increased in four patients, the highest value being 206 mg/100 ml (range in controls 120–150 mg/100 ml). The cephalin fraction showed a marked increase in five of these patients, the mean value being increased 4–5 times compared with the controls. The highest value was 55 mg/100 ml compared with a range of 4–14 mg/100 ml in controls.

A slight increase in the lysolecithin fraction was observed in most of the patients, an observation which needs more extensive investigation. The level of sphingomyelin in serum was not significantly increased in this group of diabetics.

The percentage distribution of the individual

(described in case reports as I, II, III, IV) showed a lipoprotein pattern consistent with a hyperlipaemia Type II according to the classification of Fredrickson *et al.* [5]. These four patients had a cholesterol level in serum ranging from 330–440 mg per 100 ml. Patient R. H. (case report III) had also a pronounced elevated pre-beta-lipoprotein fraction. The classification of this patient will therefore be consistent also with a Type III pattern. The decision whether this patient belongs to Type II, III or IV cannot be made by simple electrophoresis and routine lipid determinations, but must be confirmed by ultracentrifugation of serum and immunological investigation as discussed by Seidel *et al.* [20]. The two remaining patients showed normal distribution of these lipid constituents.

Discussion

The statement made by Fredrickson [5] that hyper-beta-lipoproteinaemia of Type II is a major causal factor in the accelerated atherosclerotic vascular disease seems well documented by many authors. In man the beta-lipoproteins are the main cholesterol and phospholipid bearing proteins. The correlation between coronary heart disease and phospholipids has been established by Nothman and Proger [15]. Their group of patients showed, in addition to hypercholesterolaemia, an increase in total phospholipids in platelet-poor plasma. An increase in the cephalin fraction was found, which amounted to 2–3 times the values found in normal controls. The cephalin fraction was also increased compared with normals when expressed as percentage of total phospholipids. Therefore these authors have called attention to the importance of this increase in the cephalin fraction in blood where arterial occlusion is present.

In the present communication the selected group of diabetic patients has shown an increase in total phospholipids, and an absolute and relative increase in the cephalin fraction. It is tempting to assume that lipoprotein particles, especially of the beta-lipoprotein type, which are rich in phospholipids, can contribute cephalins of importance for the coagulation process in blood. Several observations are in line with this hypothesis. Thus O'Brien [16] has found that phosphatidylethanolamine could substitute for platelets in the thrombin generation test, and Stypven clotting time, whereas lecithin was almost completely inactive in the coagulation of human plasma poor in platelets. Chylomicrons have been used as a source of cephalin in thrombin generation test [18], and shortening of clotting time can be observed under alimentary lipaemia. If, as suggested by Poole [17], FFA in blood also can contribute to the shortening of the clotting time, at least part of this effect can be attributed to the lipoprotein particles produced in the liver in response to FFA. Kline [11] has pointed out that even liver microsomes can have pro-coagulant activity, which when liberated to the blood can induce prothrombin activity.

The mechanism of liberation of cephalin into the blood or in the blood is poorly understood. The release of phospholipids may be related to the action of ADP [3], which is known to bring about an adhesion and aggregation of platelets [8]. Such a mechanism may be operating in disease where increase in the concentration of the cephalins in the blood is observed. Since the present work has shown an increase in total phospholipids with a relative increase in the cephalin fraction, the methylating capacity of the liver and the fat secretion mechanism are most likely disturbed under the pathological conditions described.

An important problem is whether or not the abnormalities in serum phospholipids are of primary significance for the late complications in diabetes. In

studies published elsewhere [25] we have observed that diabetic patients under good clinical control have normal serum lipoprotein levels including those patients who had developed late manifestations. There was no relationship between the level of pre-beta-lipoproteins and diabetic retinopathy or neuropathy. Hyperlipoproteinaemia was present only in juvenile diabetics with ketoacidosis. The concept that hyperlipoproteinaemia in diabetics is related to poor control of the disease is supported by this and other investigations [1, 9, 19]. In agreement with these authors, we have not observed hyperlipoproteinaemia or an increase in serum cephalin or lecithin in maturity-onset diabetics in which there was good clinical control (unpublished observations). As mentioned previously, an appropriate metabolic control of the diabetic patients reported in the present study had not been achieved. Our investigations, therefore, indicate that the abnormalities in serum lipid constituents which have been observed occur as a secondary consequence of the diabetic metabolic disturbances.

It should be pointed out that increased serum cephalin is not a common finding in non-diabetic patients with hyperlipoproteinaemia. In such patients Vikrot *et al.* [23] and Wille and Phillips [26] observed disturbances in the sphingomyelin/lecithin ratio but no increase in serum cephalin.

In conclusion, this study indicates that hyperlipoproteinaemia with an absolute and relative increase in serum cephalin occurs as a consequence of insufficient metabolic control in diabetic patients. Although our evidence indicates that these disturbances do not cause the late-developing manifestations of diabetes, these may be exaggerated when the serum cephalin level is increased.

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