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HYPERLIPOPROTEINAEMIA IN PONIES: MECHANISMS AND RESPONSE TO THERAPY

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Summary

(I) The lipid and lipoprotein concentrations in sera of 4 healthy and 9 hyperlipaemic patients were determined. From the results of the analyses, it is suggested that three types of hyperlipoproteinaemia can be distinguished in ponies:

(a) Type 1, characterised by a very pronounced increase in only the very low density lipoproteins (VLDL);

(b) Type 2, in which there is a pronounced increase in the chylomicrons (Chylo) and the very low density lipoproteins (VLDL);

(c) Type 3, which can be the result of fasting and is characterised by a moderate increase in the concentration of chylomicrons and a greater increase in very low density lipoproteins.

It is suggested that hyperlipoproteinaemia in ponies can be classified on the basis of electrophoretic patterns.

(II) To gather more information on the metabolism of extra offered lipids, blood from ponies suffering from two different types of hyperlipoproteinaemia was transfused into normal ponies and the changes in lipid and lipoprotein concentrations in the blood serum were followed. The reactions were different. When heparin was used as anticoagulant a great increase in chylomicrons and a significant increase in very low density lipoproteins in a healthy pony was observed. When sodium citrate was used as anticoagulant a clear increase in the VLDL fraction only was noticed.

Abbreviations: Lipoproteins: LP, lipoproteins; Chylo, chylomicrons; VLDL, very low density lipoproteins = pre-β-lipoproteins; LDL, low density lipoproteins = β-lipoproteins; HDL, high density lipoproteins = α-lipoproteins.

Lipids: TL, total lipids; tot C, total cholesterol (= CE/1.7 + C); PL, phospholipids; C, free cholesterol; CE, cholesterylesters; TG, triglycerides; DG, diglycerides; FFA, free fatty acids.

(III) During treatment with glucose, galactose and insulin of two ponies affected with the same type of hyperlipoproteinaemia it was found that the lipoprotein and lipid concentrations in the blood of both ponies changed in a corresponding way and became normal. Both ponies subsequently recovered.

Introduction

In a previous study, Schotman and Wagenaar [1] described disturbances in fat metabolism in ponies and found normal lipid and enzyme values in the blood serum of clinically healthy ponies. Furthermore, they concluded that hyperlipoproteinaemia in ponies is initiated by a failure of ponies to eat which results in the mobilization of endogenous fat. This leads to more than a 50-fold increase in blood serum triglyceride (TG) values and to a fatty liver. Hyperlipaemia in ponies is a disorder with a very poor prognosis; about 65% of the patients die [2—6]. By means of paper electrophoresis Schotman [5] found that there was an enormous increase in total β-lipoprotein (β-LP) concentration in the blood of hyperlipaemic ponies (at that time a separation between β- and pre-β-LP could not yet be achieved).

Bartley [7] noted in 1970 that the lipoprotein that was increased in hyperlipaemia was VLDL (pre-β-LP).

The investigations described in this paper were aimed at providing further data on changes in the lipoprotein spectrum in ponies suffering from hyperlipoproteinaemia. Because of differences in response to therapy we expected different types of hyperlipoproteinaemia as was found by Frederickson and Lees [8] in connection with human hyperlipoproteinaemia. Morris et al. [9] described changes in blood serum lipoprotein patterns in ponies with only a very mild form of hyperlipoproteinaemia induced by fasting. However, lipoproteins were isolated by ultracentrifugation in such a manner that chylomicrons and VLDL were not separated. Upon analysis of the lipids from the LP fractions, they noted remarkably low cholesterylesters to total cholesterol (CE/total C) ratios. In our study, chylomicrons, VLDL, LDL and HDL were isolated as separate fractions by preparative ultracentrifugation technique as well as by very good correlating paper electrophoretic separations.

Because hyperlipoproteinaemia in ponies also develops during fasting [16], Schotman [5] suggested that hyperlipoproteinaemia was the result of an increased mobilisation of lipids. An enormous offer of mobilised lipids should cause the observed shifts in the lipoprotein spectra of lipaemic ponies.

To gather additional information on the metabolism of extra offered lipids in our study, lipaemic blood from two sick ponies was transfused into two healthy ponies. As is known heparin activates the lipoprotein lipase [5,10]. Therefore in the blood coming from patient 1, heparin was used as anticoagulant. In the blood of the other pony sodium citrate was used as anticoagulant.

Finally, we gathered some data during a treatment with glucose, galactose and insulin of two ponies that were suffering from hyperlipaemia, to define the influence of this treatment on the lipoprotein spectrum.

Materials and Methods

(A) The animals for the typing of hyperlipoproteinaemias

To obtain data on clinically normal animals, according to the criteria of Schotman and Wagenaar [1], blood was collected from four Shetland ponies in foal. They all had been under the regular control of veterinary clinicians for a considerable period of time, and were normally fed. The patients were seven sick ponies which had been referred to the Clinic for Large Animal Medicine and were found to have lipaemic serum on first examination. Patient 1 had steatitis and a worm infection, patient 2 was emaciated probably as a result of strangles, patient 3 had colic, patient 4 was pregnant and had nothing seriously wrong, patient 5 had had a lochiometra, patient 6 had a peritonitis traumatica, patient 7 showed a worm infection and patient 8 suffered from salmonellosis. All patients had a very poor appetite at the onset of the treatment. Also included was a pregnant Shetland pony which had induced hyperlipoproteinaemia as a result of 13 days of forced fasting. The animal was clinically normal at the onset of the fasting period and in the 8th month of gestation. With the exception of two animals all ponies died in spite of treatment.

(B) The animals for the blood transfusions

The two normal ponies, acceptors, were about 8 months pregnant. Both animals belonged to the Clinic for Large Animal Medicine and were examined regularly by their veterinarians. Both ponies belonged to the above-mentioned group of normal ponies.

The hyperlipaemic ponies (donors). Donor 1 was a pony that was referred to the clinic as a patient suffering from a worm infection, a severe acidosis and hyperlipoproteinaemia (type 2). Donor 2 was the above-mentioned pregnant Shetland pony, suffering from hyperlipaemia (type 3) as a result of forced starvation for 13 days.

Transfusion 1

After adjusting the pH of the blood by bicarbonate infusion, 2.7 litres blood were collected using heparin as anticoagulant (2 mg/ml blood). Immediately after collection, this blood was transfused into acceptor 1 during a time interval of 30 min. Blood samples were taken for analysis just before transfusion and 15 min, 30 min, 1, 2, 3, 4, 6 and 12 h after the transfusion was completed.

Transfusion 2

Three litres of blood were collected using sodium citrate as anticoagulant (10 mg/ml blood) and the blood was transfused into acceptor 2 during a time interval of 24 min. Blood samples were taken for analysis just before transfusion and 1, 3, 6, 8, 12, 22, 30, 48 and 72 h after the transfusion was completed. After the transfusions were completed both acceptor ponies ate normally.

The animals for the experiments during treatment (patients)

Patient I was a 6-month-old Shetland pony suffering from hyperlipopro-

teinaemia only and presented as a patient at the Clinic for Large Animal Medicine. This patient was in a poor condition. Patient II was a 7-year-old Shetland pony suffering from hyperlipoproteinaemia. This pony seemed to be in a fairly good condition when presented to the Clinic. Colic was diagnosed. At acceptance both ponies refused to eat; both ponies subsequently recovered. The treatment with glucose, galactose and insulin was as described previously [11]. During the therapy water, hay and food concentrates were available.

Preparation of blood serum

An 8—10 ml sample of blood was collected in a 15 ml centrifuge tube, left at room temperature for 30—60 min and centrifuged at 3000 × *g* for 10 min. When the serum was to be stored for a longer period, 1 drop of a 5% solution (w/v) of merthiolate was added and the serum stored at 4°.

Fractionation of lipoproteins by preparative ultracentrifugation

The fractionation of blood serum lipoproteins was achieved by a combination of ultracentrifugation* and precipitation [12]. The LDL fraction and the combined HDL and albumin bound free fatty acid fraction were separated by means of heparin—$MnCl_2$ precipitation according to Burnstein and Samaille [13]. The HDL and the albumin bound FFA were not separated. The lipids present in the various lipoprotein (LP) fractions were extracted with methanol—chloroform (1 : 2, v/v), purified by partition according to Folch et al. [14], and determined gravimetrically after evaporation of solvent.

LP electrophoresis

Paper electrophoresis was carried out exactly according to Lees and Hatch [15].

Lipid analysis

Analysis of the lipids was performed by quantitative thin-layer chromatography [16].

The lipid composition was measured by densitometry with the exception of the phospholipids which were estimated directly in the lipid extract by colorimetric determination of phosphorus [17]. The methods of estimation of the other blood constituents mentioned in this article were as previously described [4].

For convenience many data in the tables are rounded off.

Results

From Table I, last column, it appears that in healthy ponies the distribution of the total lipoprotein lipids over the four commonly recognized LP fractions shows a remarkably constant pattern**; about two-thirds of all serum lipids are present as HDL and the remainder is almost equally divided between VLDL and LDL.

* Swing out rotor, 3 × 5 ml, No. 2414; M.S.E. SS50 centrifuge.

** Chylo, range 1—3 weight %; LDL, range 16—18 weight %; VLDL, range 10—19 weight %; HDL, range 62—72 weight %.

TABLE I

LIPOPROTEINS IN SERA OF 9 HYPERLIPAEMIC PONIES

First value (upper line) in mg/100 ml and second value (lower line, in parentheses) in weight % of total lipids.

	Hyperlipaemic ponies									Normal ponies: Mean value ABCD
	Group 1			Group 2			Group 3			
Pony:	1	2	3	4	5	6	7	8	9*	
Chylo	7	11	40	1159	2732	1472	297	745	538	8
	(1)	(2)	(3)	(36)	(45)	(49)	(6)	(19)	(14)	(2)
VLDL	400	336	947	2581	2853	992	4214	3058	2995	59
	(55)	(60)	(70)	(50)	(47)	(33)	(85)	(78)	(78)	(16)
LDL	44	107	95	361	364	301	149	39	38	64
	(6)	(19)	(7)	(7)	(6)	(10)	(3)	(1)	(1)	(17)
HDL	277	107	271	361	122	240	297	79	269	243
	(38)	(19)	(20)	(7)	(2)	(8)	(6)	(2)	(7)	(65)
Total lipids in mg/100 ml	728	560	1353	5162	6071	3005	4957	3921	3840	374

* Pony 9 was suffering from hyperlipoproteinaemia as a result of fasting for 13 days.

TABLE II

LIPIDS IN SERUM OF 9 HYPERLIPAEMIC PONIES

First value (upper line) in mg/100 ml and second value (lower line) in weight % of total lipids.

	Group 1			Group 2			Group 3			Normal ponies ABCD (range)
	1	2	3	4	5	6	7	8	9	
PL	116	140	298	723	—	631	1041	588	576	160
	(16)	(25)	(22)	(14)	(—)	(21)	(21)	(15)	(15)	(43) (38—46)
C	72	56	108	516	—	270	446	353	346	22
	(10)	(10)	(8)	(10)	(—)	(9)	(9)	(9)	(9)	(6) (5—7)
CE	138	101	216	413	—	391	347	157	230	128
	(19)	(18)	(16)	(8)	(—)	(13)	(7)	(4)	(6)	(34) (32—36)
TG	277	230	636	3097	—	1593	2924	2745	2573	41
	(38)	(41)	(47)	(60)	(—)	(53)	(59)	(70)	(67)	(11) (9—14)
FFA	124	34	95	413	—	120	198	78	115	22
	(17)	(6)	(8)	(8)	(—)	(4)	(4)	(2)	(3)	(6) (4—10)
TL	728	561	1353	5162	6071	3005	4957	3921	3840	373
tot C*	155	116	245	774	—	511	644	431	499	98
	(21)	(21)	(18)	(15)	(—)	(17)	(13)	(11)	(13)	(26)
$\frac{CE}{C}$	1.9	1.8	2.0	0.8	—	1.4	0.8	0.5	0.7	5.8
$\frac{TG}{tot\ C^*}$	1.7	1.9	2.6	4.0	—	3.0	4.5	6.4	5.1	0.4

* tot C equals $\frac{CE}{1.7}$ + C.

TABLE III

LIPID COMPOSITION IN HYPERLIPAEMIC PONIES

The lipid composition in weight % of the different lipoprotein fractions in the blood of patients 1—3 with light to moderate hyperlipaemia and of the patients 7—9 all with moderate increase in chylomicron and strong increase in VLDL concentrations and of a clinically normal pony C.

	Pony 1 (T.L. 728 mg/100 ml)						Pony 2 (T.L. 561 mg/100 ml)					Pony 3 (T.L. 1353 mg/100 ml)				
	CE	C	DG	TG	PL	FFA	CE	C	TG	PL	FFA	CE	C	TG	PL	FFA
Chylo	15.8	4.1	4.8	34.8	8.3	31.7	10.4	8.0	46.0	17.6	18.0	5.6	7.5	63.6	15.8	7.5
VLDL	21.5	5.3	12.0	44.9	12.4	3.9	3.8	12.2	64.3	17.6	2.1	4.2	9.5	67.6	15.9	2.7
LDL	8.4	18.3	5.0	18.2	23.1	27.0	36.7	11.8	25.0	21.4	5.1	43.5	9.4	15.2	17.2	14.7
HDL	11.4	14.2	2.8	17.1	21.0	33.5	30.6	6.4	4.7	34.7	23.6	34.3	6.9	2.8	27.4	28.6

	Pony 7 (T.L. 4957 mg/100 ml)					Pony 8 (T.L. 3921 mg/100 ml)					Pony 9* (T.L. 3840 mg/100 ml)					Normal pony C (T.L. 455 mg/100 ml)				
	CE	C	TG	PL	FFA	CE	C	TG	PL	FFA	CE	C	TG	PL	FFA	CE	C	TG	PL	FFA
Chylo	4.3	8.3	62.9	21.3	3.2	3.1	6.7	75.5	13.5	1.2	4.9	8.9	72.3	11.9	2.0	—	—	—	—	—
VLDL	3.4	9.3	71.7	13.0	2.6	4.0	8.2	72.5	13.2	2.1	4.2	8.8	70.7	14.1	2.2	9.6	10.7	59.8	14.1	5.8
LDL	14.8	12.9	35.4	17.1	19.8	12.7	7.6	28.8	14.1	36.8	25.0	8.2	30.2	20.0	16.6	41.7	12.8	11.1	32.0	2.4
HDL	25.6	7.7	11.9	25.5	29.3	11.0	5.0	10.7	19.3	54.0	33.1	6.6	5.4	28.6	26.3	36.3	6.9	1.4	51.8	3.6

* This pony was suffering from hyperlipoproteinaemia as a result of fasting for 13 days.

As can be seen from Table II, last column, the distribution of the total lipids over the main lipid classes shows a fairly constant picture in healthy animals; phospholipids (PL) and TG together make up 54% of the total serum lipid content.

The composition of the lipids in the LP fractions of the normal pony C is given in Table III.

We find FFA in all fractions, indicating that they are not all albumin bound (and would be concentrated only in the HDL fraction, see Methods).

(A) Hyperlipaemic ponies (the patients)

In Table I are summarised the LP-distributions in the serum of patients which had lipaemic serum at first examination. Total lipoproteins (lipids) are only slightly elevated in patients 1 and 2, moderately in 3 and strikingly so in ponies 4—9. Most pronounced is the increase in chylomicrons, hardly present in the normal state and increasing to over 45% of the total lipids in severe hyperlipaemia (patients 5 and 6). The absolute values are clearly elevated in patients 4—6. Only in patients 1—3 are the concentrations of chylomicrons within acceptable limits.

In all of the hyperlipaemic sera, the VLDL concentrations are more than 5 times as high as the values in the reference sera; in the more severe cases (patient 7), VLDL concentration increases up to 70 times. In only three cases (patients 4—6), is the concentration of LDL moderately increased.

The HDL concentration is not increased at all, but in patients 2, 5 and 8, a clear decrease is found. If we compare LP distributions, we observe a fall in HDL from 65% (mean normal value) to 2% in patients 5 and 8.

The distribution and concentrations of the different lipids are summarized in Table II. All lipid classes are elevated, but TG is most strongly affected, a 50-fold increase from 40 mg/100 ml to 2000 mg/100 ml being quite common. The increase in CE (from about 128 mg/100 ml to, at the most, 413 mg/100 ml) is far less than the increase in C from 22 mg/100 ml to, at the most, 516 mg/100 ml. In patient 8 especially the CE concentration is extremely low. From the triglyceride, cholesterylester and cholesterol values some rations (viz. C/CE and TG/total C) are calculated, in order to search for some characteristics in the lipid distributions.

From our former results in therapy, we have the impression that one might distinguish different types of hyperlipoproteinaemia in ponies. Therefore, in Tables I and II our patients are divided into three groups in order to see whether any specific differences are evident.

In group 1 (patients 1—3), with slightly to moderately elevated TL levels, over 55% of the TL is found in the VLDL fraction, the absolute levels being moderately elevated. Chylomicron levels are hardly elevated.

In group 2 (patients 4—6), either the chylomicrons and the VLDL fractions are very highly elevated in a relative as well as in an absolute sense; the LDL fractions are moderately elevated.

In group 3 (patients 7—9), the VLDL fraction comprises over 78% of the total lipids; the chylomicron fraction being moderately elevated in terms of weight % of total lipids.

Furthermore, in Table III the lipid compositions of the fractions of hyper-

lipaemic sera are compared with those of normal serum (pony C). As already mentioned FFA would be expected to be specifically present in the HDL fraction as a result of the method of fractionation, but FFA are found in all fractions. The percentage of PL is low in the LDL and HDL fractions of the serum of the patients 1—3.

Noticeable also is the rather high percentage of CE in the chylomicron fractions in ponies 1 and 2. The same phenomenon has been reported for chylomicrons in fasting human sera [12].

In the serum of patients 7—9 we found that all the chylomicrons and VLDL fractions have a high percentage of TG as usual, although the LDL fractions (3, 1 and 1% by weight of the total serum lipids) also have an elevated percentage of TG. The phospholipid content of the HDL is decreased.

Electrophoretic separation of lipoproteins. Very clear lipoprotein patterns were obtained by means of paper electrophoresis. The electrophoretic patterns were in excellent agreement with the above-mentioned figures of ultracentrifugal analysis. Chylomicrons are concentrated in bands very well separated from pre-β-LP.

(B) Transfusions

Transfusion 1. In Table IV, the lipoprotein values after transfusion are presented. Shortly (15 min) after completing the transfusion, we observed a strong rise in the VLDL fraction, a strong rise in the HDL fraction (expected because of the increase in albumin bound FFA in this fraction due to the fractionation procedure), and hardly any change in the chylomicron concentration.

Up to 12 h (the maximum time interval during this study) we find a steady and very strong increase in this chylomicron concentration and after a strong drop between 15 and 30 min a steady increase in the VLDL concentration, the HDL fraction returning to normal values. In the time interval of 0—6 h, a definite increase in LDL concentration can be observed. In Table V the

TABLE IV

LIPOPROTEIN COMPOSITION IN SERUM OF PONY A AFTER TRANSFUSION OF 2.7 LITRES OF BLOOD FROM PONY 1 (ACIDOSIS AND HYPERLIPOPROTEINAEMIA)

First value (upper line) in mg/100 ml and second value (lower line, in parentheses) in weight % of total lipids.

Time after transfusion	Donor 1	Acceptor 1	15 min	30 min	1 h	2 h	3 h	4 h	6 h	12 h
Chylo	2725	6	18	268	179	228	581	366	580	851
	(45)	(2)	(1)	(20)	(14)	(18)	(45)	(32)	(47)	(53)
VLDL	2868	57	624	176	192	127	364	196	270	444
	(47)	(19)	(42)	(13)	(14)	(10)	(28)	(17)	(22)	(28)
LDL	381	48	188	540	551	408	146	331	173	108
	(6)	(16)	(13)	(40)	(43)	(33)	(11)	(28)	(14)	(7)
HDL	97	188	661	365	374	481	207	273	211	189
	(2)	(63)	(44)	(27)	(29)	(39)	(16)	(23)	(17)	(12)
TL	6071	299	1491	1349	1296	1244	1298	1166	1234	1592

TABLE V

LIPIDS IN SERUM OF ACCEPTOR 1 AFTER TRANSFUSION OF 2.7 LITRES OF BLOOD FROM DONOR 1 (ACIDOSIS AND HYPERLIPOPROTEINAEMIA)

First value (upper line) in mg/100 ml and second value (lower line, in parentheses) in weight % of total lipids.

Time after transfusion	0	15 min	30 min	1 h	2 h	3 h	4 h	6 h	12 h
CE	125	194	216	207	199	208	233	222	223
	(42)	(13)	(16)	(16)	(16)	(16)	(20)	(18)	(14)
TG	57	447	351	324	199	662	373	617	923
	(19)	(30)	(26)	(25)	(16)	(51)	(32)	(50)	(58)
C	24	164	142	155	137	104	128	136	143
	(8)	(11)	(11)	(12)	(11)	(8)	(11)	(11)	(9)
FFA	21	507	445	453	547	78	257	86	64
	(7)	(34)	(33)	(35)	(44)	(6)	(22)	(7)	(4)
PL	72	179	189	156	162	246	175	173	239
	(24)	(12)	(14)	(12)	(13)	(19)	(15)	(14)	(15)
Total lipids	299	1491	1349	1296	1244	1298	1166	1234	1592

corresponding lipid values and some lipid ratios are summarized.

If we compare the values for 0 and 15 min, we see a strong increase in triglyceride (TG) and FFA concentration. It is striking that the FFA concentration decreases just at the moment that the TG and the VLDL concentration, but especially the chylomicron concentration, increase. The alterations in the FFA and TG concentration parallel, in general, the lipoprotein concentration, e.g., the dip in chylomicron concentration at 4 h is reflected by a dip in TG concentration.

Transfusion 2. A totally different response is measured (Table VI). It is surprising that no greater response takes place in the time interval up to 12 h after transfusion. After an initial increase measured from around 1 h up to 8 h after transfusion hardly any change could be noted. Then an almost explosive

TABLE VI

LIPOPROTEIN COMPOSITION IN SERUM OF PONY B AFTER TRANSFUSION OF 3 LITRES OF BLOOD FROM PONY 2 (HYPERLIPAEMIA INDUCED BY FASTING)

First value (upper line) in mg/100 ml and second value (lower line, in parentheses) in weight % of total lipids.

Time after transfusion	Donor 2	Acceptor 2	1 h	3 h	6 h	8 h	12 h	29 h	30 h	48 h	52 h
Chylo	504	4	13	3	4	3	12	39	373	7	2
	(14)	(1)	(1)	(1)	(1)	(1)	(2)	(3)	(13)	(2)	(1)
VLDL	2880	40	194	34	47	51	176	719	2237	56	50
	(78)	(10)	(19)	(7)	(10)	(10)	(27)	(59)	(80)	(12)	(11)
LDL	48	89	224	111	107	118	108	111	27	105	100
	(1)	(17)	(22)	(23)	(22)	(22)	(16)	(9)	(1)	(23)	(21)
HDL	249	293	592	327	317	356	359	356	155	285	308
	(7)	(72)	(58)	(69)	(67)	(67)	(55)	(29)	(6)	(63)	(67)
TL	3681	406	1023	475	475	528	655	1225	2782	453	460

increase in VLDL concentration with a maximum at 30 h after transfusion followed. This increase was accompanied by a dip in the HDL concentration. Besides that there was a definite but relatively moderate increase in chylomicrons. After 48 h the pattern was similar to that before the transfusion.

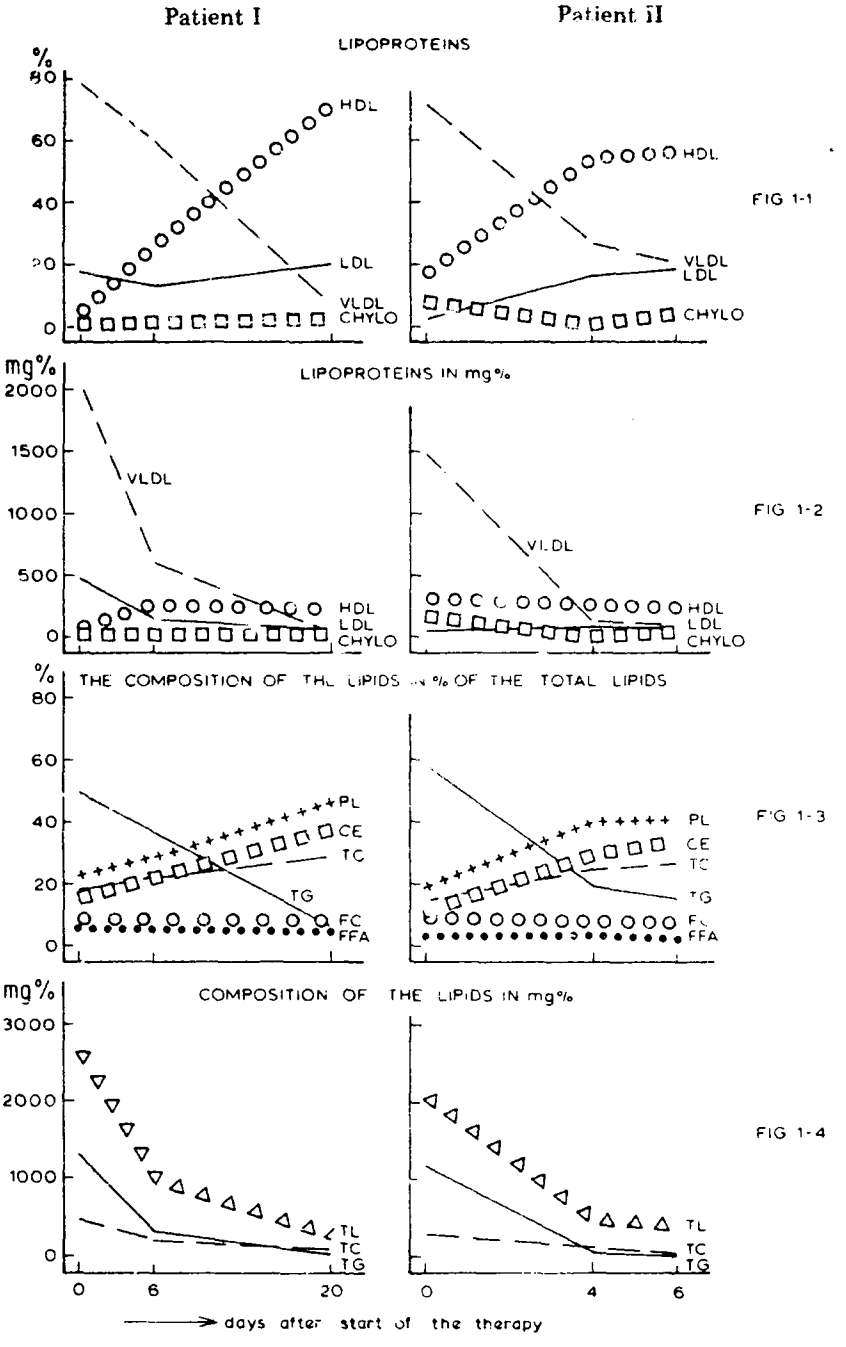

Fig. 1. The changes in the lipoprotein spectrum and in the lipid composition in percentages and in mg/100 ml in the blood of the patients I and II during treatment with glucose, galactose and insulin.

(C) The influence of treatment

The changes in lipids and lipoproteins in the blood of hyperlipaemic ponies during treatment with glucose, galactose and insulin are shown in Fig. 1. In both patients the same deviations in the lipoprotein patterns were observed, namely a slight increase of the chylomicron concentration and a clear increase only in VLDL concentration. During treatment there was a rapid decrease in VLDL and TG concentrations, but also in the glycerol and the activities of the different enzymes (AP, LDH and SDH).

Discussion

(A) The different types of hyperlipoproteinaemia

Upon consideration of the results in Table I, it is apparent that the first change in the lipoprotein pattern during the development of hyperlipoproteinaemia in ponies (patients 1—3) seems to be an increase in the VLDL concentration accompanied by a moderate rise in the TL and TG values (type 1). This change is much more pronounced in patients 7 and 8 and in patient 9 with induced hyperlipoproteinaemia (type 3); the chylomicron fraction is also somewhat elevated.

The lipoprotein concentrations in patients 4—6 (type 2) are changed in a typical way, as chylomicrons and VLDL are present in nearly equal very large amounts; the LDL concentration is also clearly elevated. It is possible that hyperlipoproteinaemia in ponies evolves from a pattern such as that in ponies of group 1 through the pattern of group 3 leading to the pattern of group 2. This seems unlikely, however, because of the specific increase in LDL concentration in type 2.

As a result of this study we may state that, in ponies, three types of hyperlipoproteinaemia may be distinguished. These are: (Type 1) a type with a predominantly strong increase in VLDL and a zero to moderate increase in chylomicron concentration (Frederickson type IV in human hyperlipoproteinaemia); (Type 2) a type in which nearly equal but highly elevated concentrations of chylomicrons and VLDL can be found (Frederickson type V in human hyperlipoproteinaemia) in association with a certain but less pronounced rise in LDL concentration; (Type 3) a type which is induced by fasting. This last type is not yet clearly established.

Clear information can be obtained by the use of paper electrophoresis according to Lees and Hatch [15] by which the two assumed types can be distinguished on the bases of an increase in VLDL only or a combined increase in VLDL and chylomicrons.

(B) The transfusions

Comparing the changes in lipoprotein concentrations after the transfusions of lipaemic blood into healthy ponies (Tables IV—VI) we observe different reactions in the blood of healthy ponies. In both experiments there is at first an increased or increasing HDL concentration, followed by a decrease in that concentration. This decrease is accompanied by a rise in the VLDL and chylomicron concentration. In the case of transfusion 1 (donor 1 → acceptor 1) the increase in total lipids in blood from acceptor A is more or less as can be

expected from mixing the sera; this total lipid value does not change very much up to at least 12 h.

The VLDL level still increases up to at least 12 h (maximal time of this experiment), accompanied by an increase in TG concentration, but a decrease in FFA concentration. The chylomicron level is unexpectedly low directly after the completion of the transfusion, but steadily increases at a high rate up to at least 12 h. The initial low values can be explained by a high turnover rate of chylomicrons as evidenced by the high FFA level (Table V).

The immediate increase in the HDL fraction (HDL + FFA) can partially be explained by taking into account the fact that there is a quick FFA release from the administered lipoproteins. This release of FFA is accelerated because the administered heparin has an activating effect on the lipoprotein lipase in the blood of the recipient pony [5]. The data concerning the course of the FFA and TG levels in Table V indicate that the greater part of this amount of FFA probably is taken up into triglycerides and finally causes a strong increase in the chylomicron and an increase in the VLDL fraction (Table IV).

The supposition that the initial increase in the HDL fraction originates from a clear increase in FFA is supported by the similarly observed decrease in the HDL fraction after 2 h. This is exactly the time that the FFA concentration is decreasing and the triglyceride concentration and the VLDL and chylomicron fractions are increasing (Tables IV and V). The response of acceptor 2, which received blood with a strongly increased VLDL level and a moderately increased chylomicron level was different. Up to 12 h after the transfusion nothing happened. However between 22 and 30 h there was a clear decrease in the HDL concentration and an explosive rise and fall from and to the normal values of VLDL. The chylomicron level increases moderately at the same time. Probably the administered lipoproteins cannot be hydrolysed at the same rate because in this case the lipoprotein lipase is not activated by heparin. Therefore, the different response could be expected. From the chylomicron and VLDL levels 12 h after the transfusions (Tables IV and VI), it can be concluded that a greater availability of FFA can lead to an increased production of chylomicrons and VLDL. The fact that the HDL fractions after the transfusions are increased until the VLDL and chylomicron fractions begin to rise suggests that the fatty acids that are released from the administered lipoproteins are gathered in the HDL fraction, probably bound to albumin. After some time these FA are used for the synthesis of TG. These TG are taken up in the chylomicrons and VLDL. The character of the changes seems to depend on the rate at which the fatty acids are released. The higher this rate of release, the more chylomicrons are synthesized. When the release of fatty acids is more gradual, then the extra offered lipids can be worked up by synthesizing more VLDL.

It is not clear to what extent observed differences between the two transfusions are influenced by the type of hyperlipoproteinaemia that occurs in the donor of the lipaemic blood.

(C) The changes occurring during treatment

In the prolonged treatment with glucose, galactose and insulin, both the patients treated appear to have suffered from liver damage, as the LDH and AP

concentrations were increased [18]. The high blood glycerol concentration that we measured in the blood of the two patients treated suggests that the hyperlipoproteinaemia occurring in these patients is the result of increased fat mobilization. The increased concentration of VLDL suggests that the same type of hyperlipoproteinaemia (type 1) was present in both patients (Fig. 1). From the changes in the glycerol concentration (Fig. 1) it is evident that the therapy leads to a decrease in fat mobilization and that the initially high TG concentration generally decreased. The influence of the therapy on the deviations in the lipoprotein spectrum is also very obvious. A decrease in the percentage of VLDL and a rise in the percentage of HDL can be observed in both patients (Fig. 1). After the mobilization decreases as a result of therapy, the removal of the lipids (mainly TG) from the liver to the different tissues and the adipocytes can apparently be effected by gradually returning to the synthesis of the normal pattern of lipoproteins (Fig. 1). The observation that the PL concentration as well as the percentage of HDL increase supports this hypothesis.

The observation that a therapy with glucose alone did not result in a decrease of the TL concentration in the blood [3,5] suggests that insulin plays an important role. One of the activities of insulin is to stimulate FFA uptake in the adipocytes [19—21]. Therefore, the explanation for the observed changes might rather be sought in a decreased fat mobilisation [22] and an increased FFA uptake in the adipocyte. Perhaps a fully normally functioning liver can still regulate the lipid transport during an extra increase in TG without a shift in lipoprotein synthesis. A liver with a large pool of endogenous triglycerides, for instance, cannot manage that [23]. Which comes first, an increased concentration of TG in the liver or the shift in lipoprotein synthesis, is not known at this time.

Summarizing, it can be stated that increased fat mobilization in ponies with slight liver damage results in a shift in lipoprotein synthesis. The greater amount of fat mobilized, the more VLDL and chylomicrons are synthesized.

Treatment of ponies suffering from hyperlipoproteinaemia with glucose, galactose and insulin can effect a decrease in total lipid concentration and a correction of the disturbed LP pattern in the blood.

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