Distribution of glycosphingolipids in the serum lipoproteins of normal human subjects and patients with hypo- and hyperlipidemias

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Abstract Five glycosphingolipids (GSL), glucosylceramide, lactosylceramide, trihexosylceramide, globoside, and hematoside (G_{M3}) were studied in serum from normal human subjects and patients with dyslipoproteinemia and found to be exclusively associated with the various classes of serum lipoproteins. Based on a unit weight of lipoprotein protein, the total amount of GSL in serum from normal subjects was twice as high in very low density lipoprotein (VLDL) (d < 1.006 g/ml) and low density lipoprotein (LDL) (d 1.019–1.063 g/ml) as in high density lipoproteins HDL_2 (d 1.063–1.125 g/ml) or HDL_3 (d 1.125–1.21 g/ml). In abetalipoproteinemia the levels of serum GSL were slightly reduced when compared to normal serum and were all found in the only existing lipoprotein, HDL; this contained 2–3 moles of GSL/mole of lipoprotein as compared to 0.5 GSL/mole in normal HDL. In hypobetalipoproteinemia and Tangier disease, the serum glycosphingolipids were 10 to 30% reduced in concentration compared to the 75% reduction in other lipids, and were again found to be associated only with the serum lipoproteins. The relative proportions of GSL did not vary substantially in the normo- and hypolipidemic subjects studied. Only in patients with homozygous familial hypercholesterolemia was there a significant (3–4-fold) elevation of all of the five GSL species and this elevation correlated well with that of the circulating cholesterol and LDL. On a molar basis the LDL of these patients contained the same amount of GSL as normal subjects (5 moles GSL/mole protein). It is concluded that: (*1*) glycosphingolipids are associated only with the major lipoprotein classes in both normal and dyslipoproteinemic serum; (*2*) the relative proportions of the five glycosphingolipids are not significantly affected by dyslipoproteinemia; (*3*) only in severe hypolipoproteinemia do the remaining serum lipoproteins carry a complement of glycosphingolipids greater than normal. Although our results establish that glycosphingolipids are intimately associated with serum lipoproteins, the mode of association or the structural and functional significance of such an association remains undetermined.

Supplementary key words serum glycosphingolipids • gas–liquid chromatography • abetalipoproteinemia • hypobetalipoproteinemia • Tangier disease • familial hypercholesterolemia • mixed hyperlipoproteinemias

The presence of glycosphingolipids (GSL) in human plasma was first reported by Svennerholm and Svennerholm in 1963 (1). They were subsequently characterized as glucosylceramide (GL-1a), lactosylceramide (GL-2a), galactosylgalactosylglucosyl ceramide (GL-3a) and globoside (GL-4a) by Vance and Sweeley (2). Tao and Sweeley (3) have since identified G_{M3} as a major sialoglycosphingolipid in plasma and the presence of trace amounts of more complex GSL containing sialic acid and GalNAc has also been reported (4, 5). These GSL have not yet been fully characterized except for the Le^a and Le^b glycolipids (6). Vance, Krivit, and Sweeley (7) demonstrated that in Gaucher's disease plasma glucosylceramide (GL-1a) levels were significantly elevated and that plasma trihexosylceramide (GL-3a) levels were consistently elevated 2- to 3-fold in Fabry's disease. Apart from these findings the determination of plasma GSL levels has not been of general value in the diagnosis of sphingolipid storage diseases such as the gangliosidoses and leukodystrophies, since galactosylceramides and complex brain gangliosides are present only as trace components in plasma.

In 1967 Skipski et al. (8) reported that glucosylceramide was associated with both the isolated lipoprotein fractions

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Abbreviations: GSL, glycosphingolipids; VLDL, very low density lipoproteins (d 1.006 g/ml); LDL, low density lipoproteins (d 1.019–1.063 g/ml); HDL, high density lipoproteins (d 1.063–1.21 g/ml), HDL_2, HDL of d 1.063–1.125 g/ml; HDL_3, HDL of d 1.125–1.121 g/ml; GL-1a, Glc-Ceramide; GL-2a, Galβ(1→4)Glc-Ceramide; GL-3a, Galα(1→4)Galβ(1→4)Glc-Ceramide; GL-4a, GalNAcβ(1→3)Galα(1→4)Galβ(1→4)Glc-Ceramide; G_{M3}, NeuNAcα(2→3)Galβ(1→4)Glc-Ceramide; ABL, abetalipoproteinemia; HBL, hypobetalipoproteinemia; HFH, homozygous familial hypercholesterolemia; GLC, gas–liquid chromatography.

TABLE 1. Human subjects studied

No.	Patient	Sex	Age	Diagnosis	Clinical Information	Serum Total Cholesterol	Serum Total Triglycerides
						mg/dl	
1	S.F.	M	20	Normal	Asymptomatic	210	70
2	D.M.	M	21			200	80
3	L.H.	F	22			220	80
4	S.T.	F	20			210	80
5	W.D.	M	24	Hypobetalipoproteinemia	Neurological manifestations compatible with Friedreich's ataxia	103	75
6	B.D.	M	12	Hypobetalipoproteinemia	Same as above but less severe	40	70
7	A.M.V.	F	16	Abetalipoproteinemia	Fat malabsorption, retinitis pigmentosa, acanthocytosis, neurological manifestations, abetalipoproteinemia	30	20
8	M.S.	M	19			45	15
9	R.I.	M	20			40	20
10	C.N.			Tangier disease	Storage of cholesterol esters in many tissues of the body. Severe deficiency or absence of normal HDL	80	190
11	M. van N	F	9	Homozygous familial hypercholesterolemia (HFH)	Both parents with heterozygous familial hypercholesterolemia, occurrence of severe hypercholesterolemia in at least one sibling, diffuse xanthomas, early vascular disease	642	109
12	B.N.	M	11			612	60
13	L. van S.	F	13			831	132
14	H. van N	F		Heterozygous familial hypercholesterolemia		282	69
15	R. van N.	M				273	51
16	T.S.	M	32	Mixed hyperlipidemias	Asymptomatic	260	285
17	S.T.	F	40			316	1392
18	L.M.	F	42			342	2340

and the ultracentrifugal residue. Other glycosphingolipids were not characterized since this study was concerned mainly with neutral lipids and phospholipids. Studies on porcine blood (9) have indicated that both plasma glucosylceramide (GL-1a) and red cell GL-1a are in dynamic equilibrium, in contrast to plasma GL-2a, GL-3a, and GL-4a which do not exchange with their erythrocyte counterparts (9). In these studies it was assumed that hydrophobic plasma glycosphingolipids were solubilized by association with protein or lipoprotein. In the present studies, we have determined the amount and nature of the GSL associated with whole serum and with the major ultracentrifugal serum lipoprotein and other serum protein fractions, both in normal subjects and in patients with dyslipoproteinemias. The results obtained are the subject of this report.

MATERIALS AND METHODS

Normal human sera were obtained from the blood of overnight-fasted male and female Caucasian donors, 20–25 years of age, type A, Rh^+. The patients with abetalipoproteinemia, (ABL) and hypobetalipoproteinemia (HBL) were those described previously (10, 11). At the time of analysis they had values of serum cholesterol and triglycerides in the same range as reported (10, 11). The serum from a patient with Tangier disease was supplied by Dr. P. N. Herbert of the National Institutes of Health, Bethesda, Maryland. The sera of patients with homozygous familial hypercholesterolemia (HFH) (12), also referred to as Type II hyperlipoproteinemia (13), were mailed by Drs. E. Stein and D. Mendelsohn from South Africa. In these cases the diagnosis was based on the following criteria: fasting serum cholesterol levels of probands between 600 and 800 mg/dl, unresponsiveness to dietary management, serum triglycerides around 100 mg/dl, parents with heterozygous familial hypercholesterolemia, one or two siblings of proband with HFH and clinical evidence of cardiovascular disease. Some subjects with mixed hyperlipidemia (elevation of both serum cholesterol and triglycerides) were also studied. The hyperlipidemia was associated with no disease state, and their genetic identification was not established. A summary of the patients involved in this study is presented in **Table 1.**

In normolipidemic sera after removal of the chylomicrons by centrifugation at 10×10^4 *g*-av/min, the major lipoproteins VLDL (d < 1.006 g/ml), LDL (d 1.019–1.063 g/ml), HDL_2 (d 1.063–1.12 g/ml), and HDL_3 (d 1.12–1.21 g/ml), and the bottom fractions of d > 1.21 g/ml were obtained by sequential preparative ultracentrifugation as previously described (10, 14). Each fraction was subjected to purification

TABLE 2. Glycosphingolipids in lipoprotein fractions from normal serum

Fraction	GL-1a	GL-2a	GL-3a	GL-4a	G_{M3}
			μmoles/dl serum		
Whole serum	0.60 ± 0.1[a]	0.45 ± 0.1	0.20 ± 0.1	0.15 ± 0.1	0.45 ± 0.1
			μmoles/g protein		
VLDL					
Male A+	2.0 ± 0.5	2.4 ± 0.8	0.4 ± 0.2	0.2 ± 0.1	3.0 ± 1.0
Female A+	2.0 ± 0.6	2.2 ± 0.9	0.9 ± 0.3	0.3 ± 0.1	3.8 ± 1.2
LDL					
Male A+	1.8 ± 0.2	2.5 ± 0.5	0.7 ± 0.1	0.5 ± 0.1	4.5 ± 0.5
Female A+	1.8 ± 0.7	2.7 ± 0.8	0.8 ± 0.2	0.6 ± 0.1	4.5 ± 0.5
HDL_2					
Male A+	1.1 ± 0.2	1.2 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	2.0 ± 0.5
Female A+	1.0 ± 0.2	1.4 ± 0.2	0.6 ± 0.2	0.2 ± 0.2	2.0 ± 0.5
HDL_3					
Male A+	1.1 ± 0.3	1.0 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	1.6 ± 0.3
Female A+	1.1 ± 0.2	1.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	1.5 ± 0.2

[a] Mean ± SEM, four subjects.

by ultracentrifugal washing procedures (10, 14) to maximize purity of the materials. After these purification steps, there was no immunological cross-reactivity between LDL and HDL preparations, nor was albumin detected except for the d > 1.21 g/ml bottom fractions. By 1% agarose gel electrophoresis, VLDL, LDL and the two HDL had pre-β, β, and α mobility respectively.

The same procedures followed for the normolipidemic subjects were carried out for the HFH patients, the Tangier (13) serum and the cases of mixed hyperlipidemia. The separation of the serum lipoproteins of patients with ABL and HBL has been reported previously (10, 11).

Before use, the lipoproteins or the ultracentrifugal d > 1.21 g/ml bottom fractions were dialyzed against 0.15 M NaCl, 0.05% EDTA, pH 7.2. Some of the samples were delipidated by the ethanol–ether procedures outlined by Scanu and Edelstein (15), and lipid extracts were examined for total cholesterol (16) and triglyceride (17) content.

Glycosphingolipids were isolated by extraction of serum (2–20 ml) or of isolated lipoprotein with chloroform and methanol according to the procedure of Folch, Lees, and Sloan Stanley (18) as modified by Vance and Sweeley (2). After aqueous partition of the filtered extract (chloroform–methanol–0.9% KCl, 2:1:0.6), the total lipid extract (lower phase) was fractionated by a modification (19) of the Vance and Sweeley (2) silicic acid procedure. Individual GSL were separated by thin-layer chromatography on pre-coated plates of Silica gel G (Analtech, Inc., Newark, Del.) as described previously (19). The upper aqueous phase of the partition was examined in a similar manner for ganglioside content (19), and individual GSL were identified by their R_f value on thin-layer chromatography and quantitated by gas–liquid chromatography (GLC) of their derived trimethylsilyl ethers (2, 19). For example, G_{M3} was identified by the presence of Gal, Glc and NeuNAc in the ratio 1:1:1. The values for G_{M3} are the sum of materials isolated from both the upper and the lower phases of the Folch (18) partition. Phospholipid content of serum and isolated lipoprotein fractions was determined as described previously (20).

The protein content in each lipoprotein fraction was determined according to the procedure of Lowry et al (21), with bovine serum albumin used as the standard. The individual GSL were identified on the basis of their comigration with authentic standard GSL on thin-layer chromatography and from the ratio of individual sugars. The authenticity of this type of identification has been well documented previously (2–5, 7, 19).

TABLE 3. Distribution of glycosphingolipids between lipoprotein fractions[a] in female human A+ serum

GSL	Serum	VLDL[b]	LDL[c]	HDL_2[d]	HDL_3[e]
	μmoles/dl		*% of total serum GSL*		
GL-1a	0.6	13	59	14	14
GL-2a	0.4	14	60	14	12
GL-3a	0.2	12	62	13	12
GL-4a	0.15	12	64	12	12
G_{M3}	0.55	14	60	14	12
Total (μmoles/dl)	1.9	0.2	0.9	0.3	0.4

[a] The ultracentrifugal residue contained less than 1% of the serum GSL.
[b] VLDL concentration in serum is 159 ± 35 mg/dl, comprising 8% protein, 70% neutral lipid and 22% phospholipid.
[c] LDL concentration in serum is 303 ± 36 mg/dl, comprising 21% protein, 57% neutral lipid and 22% phospholipid.
[d] HDL_2, concentration in serum is 172 ± 81 mg/dl, comprising 43% protein, 18% neutral lipid and 29% phospholipid.
[e] HDL_3 concentration in serum is 264 ± 59 mg/dl, comprising 56% protein, 21% neutral lipid and 23% phospholipid.

RESULTS

Glycosphingolipids in normal lipoprotein fractions

Human plasma or serum and each of the major lipoprotein classes, namely VLDL, LDL, HDL_2, and HDL_3, contained all of the five major plasma GSL: glucosylceramide (GL-1a), lactosylceramide (GL-2a), trihexosylceramide (GL-3a), globoside (GL-4a) and hematoside (G_{M3}) in approximately the same proportion (**Table 2**). Within the limits of the GLC analytical technique (0.005 μmoles GSL) we were unable to detect GSL in the "ultracentrifuge residue" (d > 1.21 g/ml). All lipo-

TABLE 4. Glycosphingolipid composition of serum from patients with abetalipoproteinemia (acanthocytosis), hypobetalipoproteinemia (Friedreich's ataxia) and Tangier disease

Patient and Fraction	GL-1a	GL-2a	GL-3a	GL-4a	G_{M3}
			μmoles/dl serum		
	0.60 ± 0.1[a]	0.45 ± 0.1	0.20 ± 0.1	0.15 ± 0.05	0.45 ± 0.1
Abetalipoproteinemia					
Patient No. 7[b]	0.44	0.33	0.17	0.10	0.52
8	0.42	0.20	0.11	0.20	0.30
9	0.39	0.29	0.06	0.22	0.35
Hypobetalipoproteinemia					
Patient No. 5	0.45	0.28	0.09	0.10	0.45
6	0.42	0.27	0.10	0.11	0.43
Tangier Disease					
Patient No. 10	0.45	0.23	0.08	0.10	0.40

[a] Mean ± SEM, six subjects.
[b] Numbers refer to patients in Table 1.

TABLE 5. Glycosphingolipid composition of lipoprotein fractions derived from patients with hypolipoproteinemias

Patient and Fraction	GL-1a	GL-2a	GL-3a	GL-4a	G_{M3}
			μmoles/g lipoprotein		
Control, HDL[a]	1.00	1.00	0.30	0.20	0.85
Abetalipoproteinemia, HDL					
Patient No. 7[b]	3.40	2.50	1.40	1.50	3.10
8	5.40	4.80	3.00	2.40	1.90
9	6.70	4.50	1.56	3.50	4.40
Control, LDL[a]	2.00	2.60	0.80	0.60	4.50
Tangier Disease, LDL					
Patient No. 10	2.30	3.60	1.25	1.30	5.50

[a] See Table 2 for range of normal values of VLDL, LDL, HDL_2 and HDL_3.
[b] Numbers refer to patients in Table 1.

protein fractions were isolated from clotted human serum and thus far we have been unable to ascertain any GSL differences between serum and plasma. When results are expressed on the basis of μmoles GSL/g of lipoprotein protein, the overall concentrations in VLDL and LDL were approximately two-fold greater than those in HDL_2 or HDL_3 (Table 2). Excluding the small amounts of galactosylceramide (GL-1b), sulfatide (GL-1bS), "uncharacterized" plasma gangliosides (of which only G_{D3} was positively identified) and fucoglycolipids, the total GSL content of VLDL and LDL from normal males was 8.6 ± 1.3 and that of HDL_2 and HDL_3 4.2 ± 1.0 μmoles/g of protein respectively. There were some GSL differences between corresponding fractions derived from male and female donors (Table 2), but their significance is not clear. The variation can be possibly attributed to both biological and methodological differences since we did not study a sufficiently large number of subjects to eliminate this possibility. The relative amounts of the five major GSL in the four lipoprotein fractions were approximately the same (**Table 3**), namely 12–14% in VLDL, 59–64% in LDL, 12–14% in HDL_2 and 12% in HDL_3 in female A^+ serum. In male serum the low levels of HDL_2 were reflected in an increased amount of GSL (65–72%) being associated with the LDL fraction. Sulfatide (GL-1bS), identified only by its R_f value on thin-layer chromatography and the presence of only Gal upon GLC analysis, was found to be preferentially associated with LDL and HDL_2 at a level of 0.5 μmoles/g lipoprotein protein. In every case, the "bottom fraction" (ultracentrifugal residue, which con-

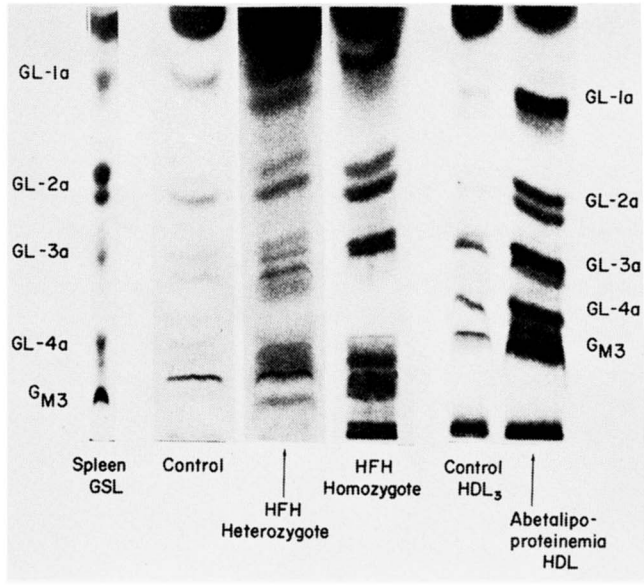

Fig. 1. Thin-layer chromatogram of the neutral glycosphingolipids; GlcCer (Gl-1a); LacCer (GL-2a); TrihexCer (GL-3a), Globoside (GL-4) and G_{M3} isolated from 4 ml of serum from control, heterozygous familial hypercholesterolemic and homozygous familial hypercholesterolemic patients, normal HDL_3 lipoprotein and HDL (the only lipoprotein fraction) from a patient with abetalipoproteinemia. The solvent system used was chloroform–methanol–water 100:40:6, and the GSL were visualized with iodine. Experimental details are described in the text.

TABLE 6. Glycosphingolipid composition of serum derived from homozygous and heterozygous patients with familial hypercholesterolemia and mixed hyperlipidemias

Patient	GL-1a	GL-2a	GL-3a	GL-4a	G_{M3}
			μmoles/dl serum		
	0.60 ± 0.1[a]	0.45 ± 0.1	0.20 ± 0.1	0.15 ± 0.1	0.45 ± 0.1
HFH homozygotes					
Patient No. 11	2.79	1.39	1.01	0.60	2.79
12	1.77	1.44	0.71	0.57	1.79
13	2.38	1.56	1.19	0.69	2.51
HFH heterozygotes					
Patient No. 14	0.79	0.70	0.29	0.24	0.75
15	1.03	0.62	0.27	0.27	0.87
Mixed hyperlipidemias					
Patient No. 16	1.18	0.71	0.23	0.27	0.70
17	0.60	0.42	0.12	0.07	0.55
18	0.84	0.47	0.13	0.10	0.55

[a] Mean ± SEM, six subjects.

tains all the other plasma proteins such as albumin, α-globulins and the immunoglobulins) contained undetectable (<0.005 μmoles) amounts of GSL, indicating that all circulating GSL were associated with lipoprotein.

Glycosphingolipids in abetalipoproteinemia

The analysis of serum obtained from three patients with abetalipoproteinemia revealed only slightly reduced concentrations of all five GSL (**Table 4**), in contrast to the marked reduction in phospholipids, cholesterol, and triglyceride concentrations previously reported (10) (Table 1). Since there is an almost total absence of VLDL and LDL in these patients, only the HDL fraction was examined. Studies revealed a much higher concentration of GSL (expressed as μmoles/g protein) than found in normal HDL_2 or HDL_3 (**Fig. 1**) (**Table 5**). Of the individual GSL, GL-1a, GL-2a and G_{M3} were enriched 3- to 5-fold, whereas GL-3a and GL-4a showed an apparent 10-fold enrichment (Table 3). In contrast to the changes in serum GSL, erythrocyte GSL levels were found to be within the normal range (2, 4, 19) in all the patients studied.[4]

Glycosphingolipids in hypobetalipoproteinemia

Serum from two patients with hypobetalipoproteinemia (11) was analyzed and found to contain amounts of all five GSL that were slightly lower than the normal means (Table 4).

Glycosphingolipids in Tangier disease

The study of this genetic disorder has indicated a marked deficiency of HDL (13). Serum GSL levels were only slightly lower than the normal means (Table 4), with GL-2a and GL-3a showing the most striking decreases. The concentrations were comparable to those found in abetalipoproteinemic and hypobetalipoproteinemic sera and did not reflect a striking reduction in cholesterol and phospholipid levels (Table 1). Analysis of the GSL content of LDL from this patient indicated a slight enrichment compared with that of normal LDL (Table 5).

Glycosphingolipids in serum from patients with homozygous familial hypercholesterolemia

Analysis of serum from several patients with homozygous familial hypercholesterolemia (with cholesterol levels in the range of 600–800 mg/dl and triglyceride levels of 50–100 mg/dl) (Table 1) revealed a 3 to 4-fold elevation in all five major classes of GSL (**Table 6**). The increased GSL concentration was directly proportional to the increased concentration of circulating LDL cholesterol phospholipid and protein (Table 6). Heterozygous patients, who exhibited a lesser (2-fold) elevation of cholesterol, were found to have serum GSL levels slightly higher than normal (Table 6).

Glycosphingolipids in serum from patients with mixed hyperlipidemias

Some patients with elevation of both serum cholesterol and triglycerides (many of whom are probably heterozygotes for familial hypercholesterolemia (12), Type II hyperlipidemia (13)) exhibited elevated (20–30%) levels of GL-1a, GL-2a, GL-3a, GL-4a, or G_{M3}, but this was not a consistent finding. Some examples are presented in Table 6. Patients in whom cholesterol levels were normal but whose serum triglyceride levels were elevated 5 to 25-fold showed essentially no elevation in serum GSL concentration. In general GSL levels in isolated VLDL, LDL, and HDL from such patients fell within the normal range (**Table 7**). GL-2a levels tended to be below normal, especially in VLDL and HDL, whereas GL-3a levels tended to be higher in LDL and lower in HDL. No consistent pattern was observed and this may reflect the heterogeneity of the mixed hyperlipidemias.

DISCUSSION

Our results have shown that all of the serum glycosphingolipids in normal and dyslipoproteinemic subjects are associated with lipoproteins. The highest concentration of GSL, relative to protein, was found in the VLDL and LDL fractions with less in HDL_2 and HDL_3. In contrast, no GSL was

[4] Dawson, G., A. W. Kruski, and A. M. Scanu. Unpublished data.

TABLE 7. Glycosphingolipid composition of lipoprotein fractions isolated from patients with mixed hyperlipidemias

Patient and Fraction	GL-1a	GL-2a	GL-3a	GL-4a	G_{M3}
			μmoles/g lipoprotein protein		
VLDL					
Normal Male	2.0 ± 0.5[a]	2.4 ± 0.8	0.4 ± 0.2	0.2 ± 0.01	3.0 ± 1.0
Patient No. 16	2.3	1.6	0.6	0.2	2.7
17	2.1	1.5	0.4	0.2	2.5
18	1.9	1.2	0.8	0.4	3.0
LDL					
Normal Male	1.8 ± 0.02	2.5 ± 0.5	0.7 ± 0.01	0.5 ± 0.1	4.5 ± 0.1
Patient No. 16	3.70	2.34	1.54	0.87	5.50
17	3.47	2.22	0.87	0.62	4.50
18	3.08	1.42	1.01	0.81	4.75
HDL					
Normal Male	1.0 ± 0.2	1.1 ± 0.2	0.3 ± 0.01	0.2 ± 0.1	1.7 ± 0.3
Patient No. 16	1.07	0.34	0.23	0.12	1.31
17	0.64	0.21	0.19	0.08	0.79
18	0.80	0.34	0.15	0.07	0.59

[a] Mean ± SEM, four subjects.

detected in the ultracentrifugal residue that contains all the nonlipoprotein proteins. These data differ from the preliminary study of Skipski et al. (8) who found "glycolipid" (determined as monoglycosylceramide) to be predominantly associated with both HDL_3 and the ultracentrifugal residue. However, this study (8) was mainly concerned with neutral lipids and phospholipids, and no attempt was made to study the GSL in detail other than to identify the major GSL as "ceramide monoglucoside".

Of the GSL found in plasma, lactosylceramide (GL-2a; cytolipin H) is known to be a strong hapten (22), and GL-3a and GL-4a have recently been identified as the human erythrocyte P^k and P antigens respectively (23). It is possible that the antigenic properties exhibited by membrane-associated GSL could be enhanced by an association with human lipoproteins. However, the biological role of serum GSL is still problematical, and this study represents an initial attempt to establish whether glycosphingolipids are indeed associated with serum lipoproteins and, if so, just how specific the association is. Our data indicate that glycosphingolipids are closely associated with all classes of serum lipoprotein, but they do not permit any definite statement as to the nature of this association and whether or not the glycosphingolipids form an integral part of the lipoprotein molecule.

Analysis of normal serum lipoprotein fractions revealed that GSL concentrations, when expressed per gram of lipoprotein protein, were remarkably constant from patient to patient, with the levels in VLDL and LDL being about twice those in the HDL_2 and HDL_3 fractions. LDL contains about 5% bound carbohydrate (in the form of sialyloligosaccharide units, presumably linked through asparagine (24)) whereas HDL contains very little carbohydrate (24). In three types of genetically determined hypolipidemia, i.e., abetalipoproteinemia, hypobetalipoproteinemia, and Tangier disease, GSL levels were only slightly reduced in contrast to the drastic (75%) reduction in cholesterol and phospholipid content. In the three abetalipoproteinemic patients studied, this was reflected in a massive enrichment of all five GSL in the remaining HDL fraction (VLDL and LDL fractions are absent from these patients) which would be consistent with a role for lipoprotein in GSL transport. From our studies, the HDL particle appears to be able to bind a variable amount of GSL and one may conclude that either abetalipoproteinemic HDL is grossly abnormal in its capacity to bind GSL or that normal HDL is not fully saturated by GSL. For instance, if one assumes a particle molecular weight of 250,000 for HDL (average size of HDL_2 plus HDL_3), then in normal serum an average of one-half a mole of GSL is bound per mole of HDL particle. This value increases to between 2 and 3 moles of GSL per mole of HDL in patients with abetalipoproteinemia and such levels are comparable to those of the minor HDL phospholipids such as phosphatidylserine. It has been calculated (25) that HDL_2, for example, contains an average of 1 mole of phosphatidylserine, 3 moles of phosphatidylinositol, 5 mole of phosphatidylethanolamine, 21 moles of sphingomyelin and 97 moles of phosphatidylcholine/mole. It follows that the GSL content of HDL is of the same order as the minor phospholipids present in this particle, but the structural and functional significance of these quantitatively minor components remains unclear.

In patients with homozygous familial hypercholesterolemia, the 3-fold or 4-fold elevation in serum cholesterol (600 mg/dl and 800 mg/dl, respectively) was accompanied by a 3-fold or 4-fold elevation in each of the GSL components of normal LDL. Assuming a molecular weight of 2.2×10^6 for LDL (14), it can be calculated that a total of 5 moles of GSL are bound per mole of normal LDL, and this figure holds true for the LDL of homozygous familial hypercholesterolemic serum. Here again the amount of GSL compares with the amounts of minor phospholipids in this particle. It has been calculated (25) that each mole of LDL contains 9 moles of phosphatidylserine, 9 moles of phophatidylinositol, 15 moles of phosphatidylethanolamine, 175 moles of sphingomyelin, and 400 moles of phosphatidylcholine.

The nature of the association of GSL with lipoproteins cannot be clearly deduced from these studies. The fact that GSL levels were not found to be significantly affected by very high serum triglyceride concentrations would seem to suggest

that the triglyceride moiety is not involved in GSL binding. The data appear to indicate a direct association between GSL and the protein, cholesterol, and phospholipid moieties, although consideration of this being a transport phenomenon or an intimate association with profound biological implications is merely speculative at this point. We feel tht these studies, however, do suggest that further experiments, especially careful binding studies involving GSL and both purified lipoproteins and lipoprotein derivatives, will be rewarding. Such studies may eventually increase our understanding not only of the way in which GSL are bound to circulating lipoproteins but also of their mode of interaction with the outer surface of the cell plasma membrane, where GSL have been assigned a putative role as receptors and mediators of cell recognition and division.

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