Journal of Chromatography, 230 (1982) 231—252
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1271

LIPID CLASS AND MOLECULAR SPECIES INTERRELATIONSHIPS AMONG PLASMA LIPOPROTEINS OF TYPE III AND TYPE IV HYPERLIPEMIC SUBJECTS

A. KUKSIS*, J.J. MYHER and K. GEHER

Banting and Best Department of Medical Research, University of Toronto, Toronto (Canada)

and

W.C. BRECKENRIDGE and J.A. LITTLE

Toronto-McMaster Lipid Research Clinic, University of Toronto, Toronto (Canada)

(Received February 1st, 1982)

SUMMARY

As a further appraisal of lipoprotein interconversion and equilibration of lipid components a detailed examination was made of the chemical class and molecular species interrelationships among the major fasting plasma lipoprotein fractions within each of six male Type III and Type IV hyperlipemic subjects subsisting on free choice diets. The lipoprotein fractions were prepared by conventional ultracentrifugation and the lipid class and molecular species composition of the corresponding lipoprotein fractions were determined by gas chromatography of the intact glycerol esters and ceramides. In general, each lipoprotein fraction possessed a well defined lipid class composition, which was characterized by a dramatically decreasing triacylglycerol and increasing phospholipid and cholesteryl ester content, when progressing from the very low (VLDL) to the low (LDL) and high (HDL) density lipoproteins, as already established for normolipemic subjects. Likewise, the LDL_1 and LDL_2 of the hyperlipemic subjects contained about two times higher proportion of total phospholipid as sphingomyelin than VLDL and HDL. Furthermore, the sphingomyelins of the HDL fraction contained about 30% more of the higher and 30% less of the lower molecular weight species than the sphingomyelins of the VLDL. Smaller differences were seen in the molecular species composition of the phosphatidylcholines, cholesteryl esters and triacylglycerols among the corresponding lipoproteins. In comparison to normolipemic subjects analyzed previously, the hyperlipemic subjects showed greater individual variability. Despite this variability the lipid class and molecular species composition in the hyperlipemic subjects was again incompatible with the hypothesis which postulates direct VLDL conversion into LDL and HDL under the influence of lipoprotein lipase and lecithin: cholesterol acyltransferase. The main differences between normolipemic and hyperlipemic plasma were

0378-4347/82/0000—0000/$02.75

found to reside in the number of the VLDL and LDL_1 lipoprotein particles and not in their chemical composition or physical structure, or in the apparent mechanism of their metabolic interconversion.

INTRODUCTION

Several laboratories [1—4] have advanced more or less detailed models for a metabolic conversion of very low (VLDL) into low (LDL) and high (HDL) density lipoproteins via lipoprotein lipase and lecithin:cholesterol acyltransferase. The postulated, largely self-contained processes [5, 6] of cascading transformations of the lipoproteins dictate definite interrelationships among the various components of the precursor and product particles [7, 8]. We [9] have recently completed an extensive gas chromatographic examination of the precursor—product mass relationships among the major lipid classes and molecular species of the polar surfaces and neutral lipid cores of the VLDL, LDL_2 and HDL particles in individual samples of plasma from fasting normolipemic subjects on free choice diets. While the data obtained were consistent with the basic idea of VLDL degradation into LDL and HDL, significant differences were seen in their lipid composition, which suggested a much more complex series of transformations than those previously considered [1—4]. The findings were similar to those reported for normolipemic subjects subsisting on controlled experimental diets containing saturated or unsaturated fat [10].

We have sought further confirmation of the latter results in the present study of Type III and Type IV hyperlipoproteinemia subjects. In this we have been mindful of the possibility that the serious abnormalities in the production and/or clearance of the plasma lipids in the hyperlipemic subjects might show as yet unrecognized alterations in the usual relationships among the lipid classes and molecular species of different lipoprotein fractions. The results obtained confirm the complex relationships previously seen in the normolipemic subjects and suggest that the basic differences between normolipemic and hyperlipemic plasma lie in the number of the different lipoprotein particles and not in their structure or in the mechanism of their metabolic interconversion.

MATERIALS AND METHODS

Blood samples were obtained in the fasting state (12—14 h) from twelve hyperlipoproteinemic male subjects: six Type III and six Type IV (24—65 years old) at the St. Michaels Hospital Lipid Clinic, Toronto, Canada. The subjects lived at home and subsisted on their usual diets. The Type III (dysbetalipoproteinemia) and Type IV (hyperprebetalipoproteinemia) hyperlipoproteinemia condition in each subject was established on the basis of clinical history and the biochemical criteria suggested by the Lipid Research Clinics Program [11].

Isolation and characterization of lipoproteins

Lipoproteins were isolated essentially according to Hatch and Lees [12], as described in detail previously [9]. In the present instance, however, an LDL_1

(d = 1.019 g/ml) fraction was also recovered and its lipid classes and molecular species profiles determined. The identity of the different lipoprotein fractions was independently established by double immunodiffusion against rabbit anti-human albumin, anti-human LDL and HDL, as previously described [13]. The protein concentration in each lipoprotein fraction was determined by the method of Lowry et al. [14] using bovine serum albumin as standard. Preparations of VLDL and LDL_1 were extracted with diethyl ether after color development.

Dephosphorylation and isolation of lipids

Portions of the solutions of the various density fractions (equivalent to 0.1—0.2 ml of plasma) were hydrolyzed with phospholipase C in the presence of an excess of calcium chloride as previously described [9, 10]. The released diacylglycerol and ceramide moieties of the diacylglycerolphospholipids and sphingomyelins were then recovered, along with the original neutral lipids of each lipoprotein particle, by extraction with chloroform—methanol (2:1) in the presence of 200 μg of tridecanoylglycerol as internal standard. The extracts were centrifuged to break emulsions and the lipid extracts dried with sodium sulfate, evaporated to dryness and trimethylsilylated as previously described [9, 10].

Gas—liquid chromatography

The quantitative lipid profiles of the various lipoprotein particles were determined by means of an automated gas—liquid chromatography (GLC) system as described [15]. The gas chromatograph was equipped with an automatic liquid sampler, unheated on-column injector, and the system was capable of programmed heating, cooling and equilibration cycles. The separations were performed on a 50 × 0.2 cm I.D. stainless-steel column packed with 3% OV-1 (a methylsilicone polymer) on 100—120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.) using nitrogen as carrier gas in the temperature range 175—355°C. The retention times and peak areas were recorded by means of an electronic integrator on a punched paper tape. The peak identification and composition of samples was performed in relation to the tridecanoylglycerol internal standard using a modification of a commercially available computer program, and the results were expressed as mg% and as characteristic molar ratios of lipid classes, as previously described [15,16].

The carbon numbers of isolated diacylglycerols [17] and ceramides [18] were determined by GLC of the *tert.*-butyldimethylsilyl ethers. These GLC analyses were performed on a Beckman GC-4 gas chromatograph (Beckman Instruments, Fullerton, CA, U.S.A.) equipped with a 50 cm × 0.2 cm I.D. stainless-steel column packed with 3% OV-1 on Gas-Chrom Q (100—120 mesh). Alternatively the ceramide di-*tert.*-butyldimethylsilyl ethers were resolved by capillary GLC [19]. A 5-m column coated with SP2100 (Supelco, State College, PA, U.S.A.) was installed in a Hewlett-Packard 5880A gas chromatograph equipped with a level IV Terminal. The injector port was maintained at 280°C and the detector at 330°C. After a 1-min isothermal period at 270°C the oven was temperature programmed at 2.5°C/min up to a final temperature of 330°C. The column bleed was automatically subtracted using the single-column compensation mode of the terminal.

The fatty acid methyl esters of the various lipid ester classes were prepared by transmethylation with 6% sulfuric acid in methanol for 2 h (acylglycerols) and 8 h (ceramides and cholesteryl esters), respectively. The methyl esters were isolated, identified and quantitated by GLC on 10% EGSS-X (an ethylene glycol succinate silicone copolymer), as previously described [20].

Calculations

The peak areas were corrected for differences in flame ionization response and recovery of different components by means of appropriate standards. The content of phosphatidylcholines and sphingomyelins of the lipoprotein fractions was calculated on the basis of the areas of peaks C_{36}—C_{38} and peak C_{34}, respectively, using a series of corrections, as previously described [21]. The validity of this calculation was verified by isolating and determining the carbon number distribution of the phosphatidylcholines and sphingomyelins in the various lipoprotein classes in a representative series of Type III and Type IV subjects [19].

The core radii of the lipoprotein particles were calculated on the basis of the surface to volume ratio of a sphere as previously described [18, 22]. The total radii of the particles were obtained by adding the thickness of the surface monolayer, 2.0 nm [23]. The number of neutral lipid molecules in the particle cores were calculated by dividing the appropriate proportion of the core volume by the volume of the average cholesteryl ester and triacylglycerol molecules, respectively. Likewise, the number of the polar molecules in the surface shell were calculated by dividing the appropriate proportion of the total area by the cross-sectional area of the average phospholipid and free cholesterol molecules, respectively.

RESULTS

Total lipid profiles

Fig. 1A and B shows representative total lipid profiles of the VLDL, LDL_1, LDL_2 and HDL fractions of Type III and Type IV hyperlipoproteinemia subjects. In these elution patterns the various lipid subclasses are represented by their total acyl (plus 2), acyl plus sterol, or acyl plus sphingosine carbon numbers. Peak 27 represents free cholesterol and peak 30 the internal standard, tridecanoylglycerol, which has been added in equal amounts to each of the lipoprotein samples. In general the total lipid profiles for the corresponding lipoprotein classes of Type III and Type IV subjects are similar and, except for LDL_1, not unlike those previously described for normolipemic subjects [9, 10]. There are differences, however, in the cholesteryl ester/triacylglycerol ratios, as shown below. In addition, both Type III and Type IV subjects possess LDL_1 as a major plasma lipoprotein of the fasting state. This lipoprotein class possesses a total lipid profile intermediate between those of VLDL and LDL_2 (Table I).

Quantitative composition

The weight percentages of protein and lipid in the VLDL, LDL_1, LDL_2 and HDL fractions isolated from the individual Type III and Type IV subjects are

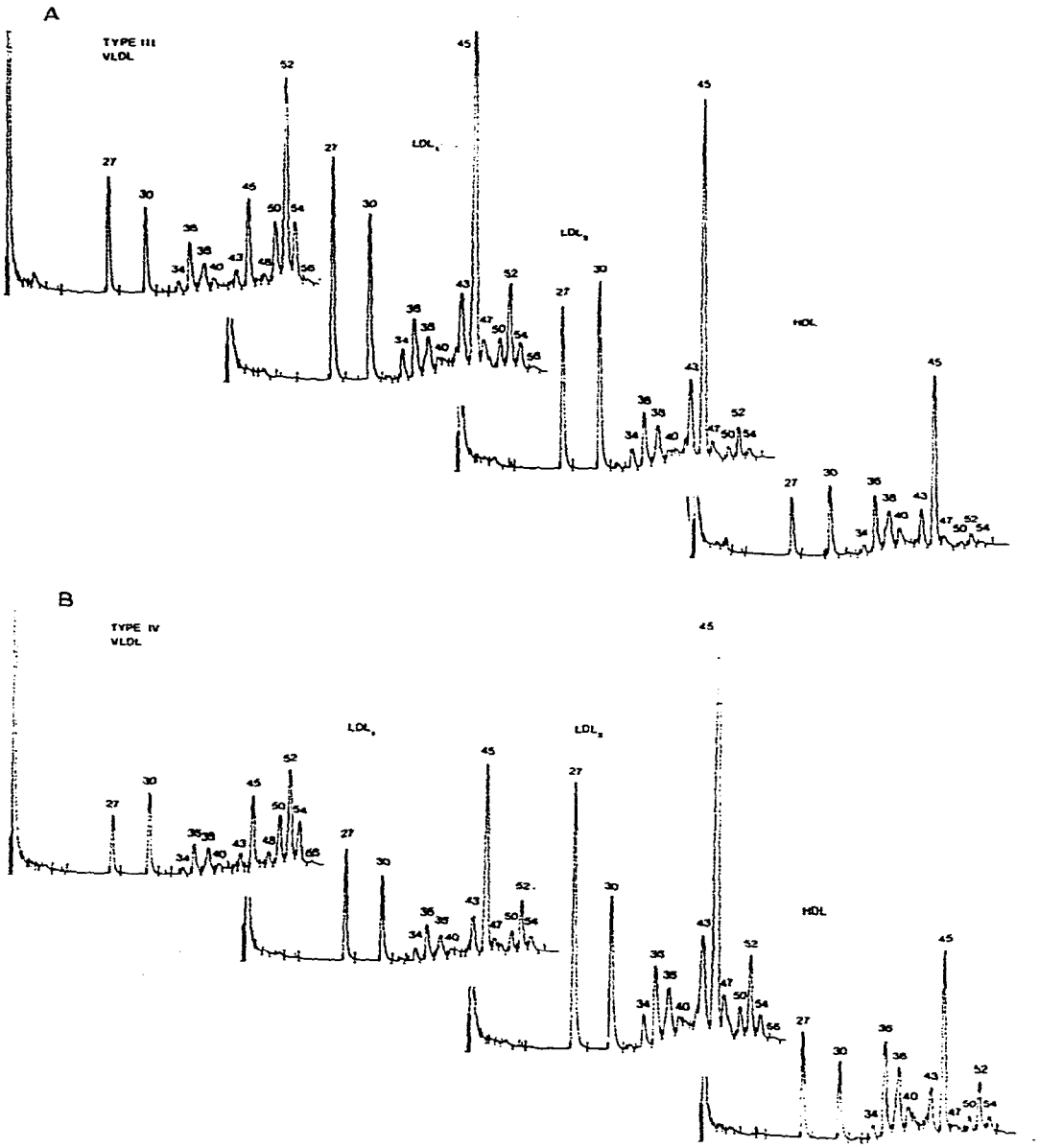

Fig. 1. GLC profiles of total lipids of plasma lipoproteins of representative subjects with Type III (A) and Type IV (B) hyperlipoproteinemia. VLDL, $d < 1.006$ g/ml; LDL_1, $d < 1.019$ g/ml; LDL_2, $d < 1.063$ g/ml; HDL, $d < 1.21$ g/ml. Conditions of high-temperature GLC as given in text. Peaks: 27, trimethylsilyl ether of cholesterol; 30, tridecanoylglycerol, internal standard; 34, trimethylsilyl ether of palmitoylsphingosine; 36—42, trimethylsilyl ethers of diacylglycerols of a total number of 34—40 acyl carbons; 43—47, cholesteryl esters of fatty acids with 16—20 acyl carbons; 48—56, triacylglycerols with a total number of 48—56 acyl carbons. Sample size: 1 μl of approximately 1% solution in silylation mixture. Attenuation: 100 times full sensitivity.

TABLE I

OVERALL COMPOSITION OF VLDL, LDL_1, LDL_3 AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

VLDL, $d < 1.006$ g/ml; LDL_1, $d < 1.019$ g/ml; LDL_3, $d < 1.063$ g/ml; HDL, $d < 1.21$ g/ml.

Chemical component*	Weight (%)												
	Type III							Type IV					
	1125	0996	1224	0973	1015	1222	Average	0745	0718	0738	0720	0011	0012	Average
VLDL													
Total protein	9	8	5	7	6	5	7 ± 2	6	5	5	6	9	9	7 ± 2
Total lipid	91	92	95	93	94	95	93 ± 2	94	95	95	94	91	91	93 ± 2
PC	20	16	13	14	13	14	14 ± 3	15	15	15	15	18	17	16 ± 1
SPH	6	3	3	3	4	2	4 ± 1	4	3	3	3	3	2	3 ± 1
CE	35	33	28	33	17	23	25 ± 9	27	14	13	18	14	11	16 ± 6
TG	29	39	49	64	61	55	50 ± 13	47	63	64	57	60	65	60 ± 7
FC	10	8	7	5	5	6	7 ± 2	7	5	5	6	5	4	5 ± 1
LDL_1													
Total protein	10	17	8	13	13	8	12 ± 2	20	14	16	17			17 ± 2
Total lipid	90	83	92	87	87	92	88 ± 1	80	86	84	83			83 ± 1
PC	17	17	18	19	19	19	18 ± 1	16	15	17	19			17 ± 1
SPH	8	9	8	6	8	7	8 ± 1	7	6	4	6			6 ± 1
CE	44	45	48	31	39	35	40 ± 2	48	37	16	37			34 ± 3
TG	21	18	15	37	25	31	25 ± 2	19	35	58	29			35 ± 7
FC	10	11	11	7	9	9	10 ± 1	10	7	5	9			8 ± 1
LDL_3													
Total protein	20	19	19	20	21	21	20 ± 1	20	26	24	18	20	19	21 ± 3
Total lipid	80	81	81	80	79	79	80 ± 1	80	74	76	82	80	81	79 ± 3
PC	22	21	21	21	23	21	23 ± 2	22	22	20	20	26	23	22 ± 3
SPH	8	7	8	7	4	5	7 ± 2	6	6	6	6	4	7	6 ± 1
CE	45	49	47	39	56	40	46 ± 6	48	50	50	57	48	54	51 ± 4
TG	16	13	14	21	10	26	17 ± 6	13	15	12	9	12	6	11 ± 3
FC	10	11	10	6	7	8	9 ± 1	11	7	10	9	10	10	10 ± 1
HDL													
Total protein	51	51	51	50	56	58	53 ± 3	68	54	40	50	58	51	54 ± 9
Total lipid	49	49	49	50	44	42	47 ± 3	32	46	60	50	42	49	47 ± 12
PC	43	40	46	41	40	43	42 ± 2	40	44	44	40	46	46	43 ± 3
SPH	5	4	5	4	4	5	5 ± 1	4	4	5	5	4	4	4 ± 1
CE	38	34	32	24	35	38	34 ± 5	36	41	35	20	31	38	34 ± 7
TG	8	17	11	27	17	10	15 ± 7	11	6	10	31	17	7	14 ± 9
FC	6	5	6	4	4	4	5 ± 1	6	5	6	4	3	4	5 ± 1

*PC, phosphatidylcholine; SPH, sphingomyelin; CE, cholesterol ester; TG, triacylglycerol; FC, free cholesterol.

given in Table I. The values for total lipids were obtained by summing the GLC estimates for the individual lipid classes, while the estimates for total protein were based on independent determinations of nitrogen on aliquots of the lipoprotein solution. It is seen that the percentage of protein in VLDL from both Type III and Type IV subjects averages 7 ± 2%, which is only slightly lower than the 9 ± 1% found previously in our laboratory for the VLDL of normolipemic subjects on free choice diets [9] but significantly lower than 12—15% noted for the VLDL of normolipemic subjects on high fat diets [19], although all of these values are within the ranges tabulated by Eisenberg and Levy [24] for VLDL of normolipemic subjects. The percentage of protein in the LDL_1 fraction from these subjects was much more variable and averaged 15 ± 4% and both Skipski [25] and Lee [26] have tabulated comparable values for this lipoprotein class from Type III and Type IV hyperlipoproteinemia subjects. The weight percentage of protein in the LDL_2 and HDL fractions averaged 23% and 53%, respectively, and corresponded to the values recorded for these lipoprotein classes in normolipemic subject [9, 10]. Therefore, these results show that the Type III and Type IV subjects in this study possess the normal protein/lipid ratios for their lipoprotein particles.

Table I also gives the weight percent composition of the major lipid classes as measured by GLC. The VLDL is seen to contain an average of 20% total phospholipid and 5—7% free cholesterol, the rest being made up of triacylglycerols and cholesteryl esters. This polar/non-polar lipid ratio is closely similar to that of VLDL of normolipemic subjects [25]. In contrast to the normolipemic subjects (EC/TG = 0.29—0.33), however, the Type III patients contained nearly twice as much cholesteryl ester and correspondingly less triacylglycerol in their VLDL (EC/TG = 0.58), while the cholesteryl ester/triacylglycerol ratio in the Type IV subjects was approximately normal (EC/TG = 0.34). Two of the six Type III patients, however, exhibited nearly normal cholesteryl ester/triacylglycerol ratios, while one of the four Type IV patients exhibited the abnormal cholesteryl ester/triacylglycerol ratio seen in Type III patients. Abnormally high cholesteryl ester/triacylglycerol ratios have previously been reported for some Type III patients by Stromberg et al. [27]. The LDL_1 and LDL_2 contained closely similar relative proportions of the different lipid classes. The total phospholipid content averaged about 30% for both Type III and Type IV subjects, while the free cholesterol ranged from 9—10%, again in close agreement with the composition of LDL_2 from normolipemic subjects. Furthermore, both groups of subjects contained about the same ratios of cholesteryl esters and triacylglycerols in these lipoprotein classes. However, in both groups some subjects possessed triacylglycerol levels which were nearly double that of normolipemic subjects for this lipoprotein class. Likewise, the HDL fraction of both Type III and Type IV patients possessed essentially normal polar/non-polar lipid class ratio, as well as a normal free cholesterol content (5%). The content of triacylglycerols was elevated (14—15%), when compared to that (5—6%) of normolipemic subjects analyzed previously [9]. There was a corresponding decrease in the content of the cholesteryl esters. Elevated triacylglycerol content in the LDL_2 fraction of Type III [28] and in the HDL fraction of Type IV [29] hyperlipoproteinemia subjects has been previously reported, but no exhaustive comparisons of the lipid class proportions have been made.

TABLE II

LIPID CLASS RATIOS IN VLDL, LDL_1, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

Chemical components*	Mole/mole											
	Type III							Type IV				
	1125	0996	1224	0973	1015	1222	Average	0745	0718	0738	0720	Average
VLDL												
FC/TC	0.18	0.20	0.24	0.20	0.15	0.26	0.20	0.32	0.35	0.40	0.36	0.36
FC/PL	0.76	0.84	0.87	0.58	0.58	0.75	0.73	0.73	0.55	0.56	0.67	0.62
SPH/PC	0.12	0.11	0.10	0.10	0.10	0.12	0.11	0.27	0.20	0.20	0.20	0.22
EC/TG	1.2	0.8	0.6	0.2	0.3	0.4	0.58	0.6	0.23	0.20	0.32	0.34
FC/SPH	3.2	5.3	4.7	3.3	2.5	6.0	4.2	3.5	3.3	3.3	4.0	3.5
FC/PC	1.0	1.0	1.1	0.7	0.8	0.9	0.91	0.93	0.7	0.7	0.8	0.78
LDL_1												
FC/TC	0.27	0.29	0.28	0.28	0.27	0.29	0.28	0.29	0.24	0.34	0.29	0.29
FC/PL	0.76	0.82	0.85	0.58	0.67	0.69	0.73	0.87	0.67	0.48	0.72	0.68
SPH/PC	0.47	0.53	0.44	0.31	0.42	0.37	0.42	0.43	0.40	0.23	0.31	0.34
EC/TG	2.0	2.5	3.2	0.8	1.6	1.1	1.9	2.5	1.0	0.3	1.3	1.3
FC/SPH	2.5	2.4	2.7	2.3	2.3	2.6	2.5	2.9	2.3	2.5	3.0	2.7
FC/PC	1.2	1.3	1.2	0.7	0.9	0.9	1.0	1.3	0.9	0.6	0.9	0.9
LDL_2												
FC/TC	0.27	0.26	0.26	0.21	0.17	0.23	0.23	0.27	0.19	0.27	0.20	0.23
FC/PL	0.68	0.78	0.68	0.35	0.51	0.61	0.60	0.78	0.50	0.77	0.70	0.69
SPH/PC	0.36	0.32	0.36	0.25	0.17	0.24	0.33	0.27	0.27	0.30	0.30	0.28
EC/TG	2.8	3.8	3.3	1.9	5.6	1.6	3.2	3.7	3.3	4.2	6.3	4.4
FC/SPH	2.6	3.1	2.5	1.8	3.5	3.2	2.8	3.7	2.3	3.3	3.0	2.8
FC/PC	0.90	1.0	0.9	0.44	0.6	0.76	0.76	1.0	0.7	0.1	0.9	0.90
HDL												
FC/TC	0.18	0.20	0.24	0.20	0.15	0.26	0.20	0.25	0.27	0.26	0.20	0.24
FC/PL	0.25	0.23	0.23	0.18	0.18	0.17	0.20	0.27	0.17	0.24	0.18	0.21
SPH/PC	0.12	0.11	0.10	0.10	0.12	0.12	0.11	0.10	0.10	0.12	0.12	0.11
EC/TG	4.7	2.0	2.9	0.9	2.0	3.8	2.7	3.3	6.8	3.5	0.6	3.5
FC/SPH	2.4	2.5	2.4	2.0	2.0	2.0	2.2	3.0	2.0	2.4	1.6	2.2
FC/PC	0.28	0.25	0.26	0.20	0.20	0.19	0.23	0.31	0.19	0.27	0.20	0.24

*TC, total cholesterol; PL, total phospholipid; other abbreviations as in Table I.

Lipid class ratios

The molar ratios of the various lipid classes in the major lipoprotein fractions of each hyperlipoproteinemic individual are given in Table II. These ratios are relatively constant and show significant differences between the Type III and Type IV subjects, and between these hyperlipoproteinemic subjects and normal subjects analyzed previously [9, 10]. Thus, the FC/TC ratio for VLDL averages about 0.20 for Type III and 0.36 for Type IV patients, while the corresponding values for normolipemic subjects average 0.44 (9, 10]. This free cholesterol occurs in a ratio of 1 molecule of sterol per 1.35 molecules of total phospholipid (0.73) for the Type III and of 1 molecule of cholesterol per 1.6 molecules of phospholipid (0.62) for Type IV patients, the normolipemic subjects approaching a ratio of 1:2 for these polar lipid components of VLDL [9, 10]. Furthermore, the Type IV subjects showed elevated SPH/PC ratios (0.22) when compared to Type III (0.11) and normolipemic (0.12—0.13) subjects [9, 10] for the VLDL fraction. The VLDL of Type III subjects possessed an increased esterified cholesterol/triacylglycerol ratio when compared to the normolipemic subjects or to the Type IV subjects, as noted above.

The lipid class ratios of LDL_2 are likewise relatively constant, but with some marked differences between the two groups of patients and between normal and hyperlipemic subjects. The FC/TC ratio in LDL_2 is the same (0.23) in both patient groups and only slightly lower than that (0.27—0.29) of the LDL_2 fraction of normolipemic subjects [9, 10]. The FC/PL ratio is about the same in both Type III and Type IV patients (0.60—0.69) and very close to that of normolipemic subjects for LDL_2 (0.70—0.72). An FC/PL ratio of 0.87—0.93, however, has been recorded for total LDL of normolipemic subjects on high fat diets [10]. The ratio of free cholesterol to sphingomyelin is the same (2.2—2.8) in both LDL_2 and HDL, while that in VLDL is significantly higher (3.5—4.2). The ratios of the other lipid classes in the different lipoproteins are similar to those in the normolipemic subjects, although there are minor differences.

Particle size distribution

The calculated particle size distribution for the various lipoprotein fractions for each subject is given in Table III. The calculated core radii for the VLDL, LDL_1, LDL_2 and HDL averaged 212, 104, 94 and 55 for the Type III and 185, 134, 93 and 53 for the Type IV, respectively. These values are closely similar for both types of subjects and are only slightly higher than the corresponding values for the VLDL, LDL and HDL particles calculated for normolipemic subjects [9]. The particle size for LDL_1 is intermediate between that of VLDL and LDL_2 as would be anticipated on the basis of the centrifugation data. Table III also includes the calculated mass of the various lipoprotein particles and demonstrates a close correspondence between the two types of patients and between these hyperlipoproteinemia subjects and normal subjects [9].

Surface and core composition

The calculated concentrations of the lipids at the surface and in the core of the VLDL, LDL_1, LDL_2 and HDL particles as the number of molecules for each of the Type III and Type IV subjects are given in Table IV. Despite considerable individual variation the average estimates for the phosphatidyl-

TABLE III

AVERAGE SIZE DISTRIBUTION OF VLDL, LDL_1, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

Parameter	Type III							Type IV						
	1125	0996	1224	0973	1015	1222	Average	0745	0718	0738	0720	0011	0012	Average
VLDL														
Core radius*	101	152	192	207	208	206	212 ± 81	164	198	198	183	169	[illegible]	185 ± 15
Particle weight**	4.3	12.7	23.3	28.8	29.1	29.2	21.2 ± 10	15.2	25.0	25.0	20.4	16.9	25.0	21.4 ± 4.6
LDL_1														
Core radius	104	95	95	123	101	107	104 ± 10	113	147	169	110			134 ± 28
Particle weight	4.7	4.0	3.6	7.5	4.5	5.0	4.9 ± 1	6.6	12.4	18.2	6.0			11 ± 6
LDL_2														
Core radius	85	88	87	86	109	110	94 ± 12	87	105	96	104	84	83	93 ± 10
Particle weight	3.2	3.5	3.4	3.3	6.1	6.2	4.3 ± 1.4	3.3	5.9	4.5	5.3	3.1	2.9	4.2 ± 1.3
HDL														
Core radius	49	60	44	62	63	53	55 ± 8	54	52	47	62	53	48	53 ± 5
Particle weight	1.3	2.2	1.0	2.2	2.7	1.9	1.9 ± 0.6	2.5	1.6	1.0	2.3	1.8	1.2	1.7 ± 0.6

*Particle radius (Å) = core radius (Å) plus thickness of outer shell (20 Å).
**Particle weight in daltons × 10^{-6}.

TABLE IV

CALCULATED CONCENTRATION OF LIPIDS AT THE SURFACE AND IN THE CORE OF VLDL, LDL_1, LDL, AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS*

Chemical component	Type III							Type IV						
	1125	0996	1224	0973	1015	1222	Average	0745	0718	0738	0720	0011	0012	Average
Surface components														
VLDL														
PC	983	2394	3632	4784	4493	4735	3504 ± 1532	2715	4501	4511	3668	3499	5138	4005 ± 874
SPH	310	471	879	1075	1460	709	816 ± 415	759	944	946	770	612	634	778 ± 144
FC	1005	2446	3997	3492	3532	4146	3103 ± 1188	2590	3067	3074	2999	1987	2471	2698 ± 433
LDL_1														
PC	911	714	753	1571	934	1095	996 ±	1073	2018	3284	1187			1890 ±
SPH	446	393	348	616	409	419	421 ±	488	840	804	390			630 ±
FC	1096	945	940	1183	904	1060	1021 ±	1371	1925	1974	1149			1604 ±
LDL_2														
PC	699	728	713	888	1412	1321	960 ± 323	742	1212	883	1088	802	690	903 ± 206
SPH	267	254	285	241	258	330	273 ± 32	212	347	278	342	129	220	254 ± 84
FC	650	780	695	403	879	1028	739 ± 213	758	788	902	1001	631	613	782 ± 151
HDL														
PC	340	538	275	576	600	424	459 ± 133	419	411	318	569	444	354	419 ± 87
SPH	40	56	30	57	61	50	49 ± 12	43	38	37	73	39	31	44 ± 15
FC	97	137	73	114	123	81	104 ± 25	129	76	89	116	59	63	89 ± 29
Core components														
VLDL														
TG	1310	5360	12577	20093	19370	17092	12634 ± 7772	7815	17370	17683	12805	10715	18048	14073 ± 4284
CE	2117	6073	9624	5465	7228	9570	6680 ± 2828	6011	5168	4810	5415	3348	4090	4807 ± 958
LDL_1														
TG	1034	695	576	2810	1129	1641	1314 ±	1170	4327	10293	1665			4363 ±
CE	2902	2328	2470	3153	2359	2481	2616 ± 334	3960	6125	3802	2844			4183 ± 1385
LDL_2														
TG	467	414	437	634	564	1502	670 ± 416	402	759	487	450	340	165	434 ± 195
CE	1760	2090	1965	1577	4232	3095	2453 ± 1019	1991	3390	2715	3814	1822	1994	2621 ± 831
HDL														
TG	58	210	60	348	234	91	167 ± 117	106	51	66	405	151	50	138 ± 136
CE	370	562	236	415	646	461	448 ± 144	464	471	312	350	369	360	388 ± 65

*Number of molecules per average particle of lipoprotein.

TABLE V

DISTRIBUTION OF MOLECULAR SPECIES OF LIPIDS IN VLDL, LDL_1, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

Carbon numbers*	Mole % of lipid class													
	Type III							Type IV						
	1125	0996	1224	0973	1015	1222	Average	0745	0718	0738	0720	L793	L794	Average
VLDL														
36	33	30	35	40	36	36	35 ± 3,3	39	37	35	35	33	40	36 ± 2,7
38	38	39	39	40	39	40	39 ± 0,8	38	41	42	40	39	39	40 ± 1,5
40	29	31	26	20	25	24	26 ± 3,9	23	22	23	25	28	21	24 ± 2,5
43	25	24	25	27	26	21	25 ± 2,1	21	24	24	28	25	24	24 ± 2,2
45 47	75	76	75	73	74	79	74 ± 2,1	79	76	76	72	75	76	76 ± 2,2
48	6	7	8	11	7	5	8 ± 2,1	8	11	10	12	5	7	9 ± 2,6
50	18	18	21	28	22	14	20 ± 4,8	19	23	24	23	17	25	22 ± 3,1
52	58	48	45	40	53	41	47 ± 7,0	43	41	43	43	57	52	46 ± 6,4
54	18	27	26	21	18	40	25 ± 8,3	30	25	23	22	21	16	23 ± 2,8
LDL_1														
36	35	33	38	40	33	43	37 ± 4,0	39	40	37	39	—	—	39 ± 1,2
38	36	40	39	41	38	44	40 ± 4,0	36	36	46	44			40 ± 5,2
40	29	27	23	19	29	13	23 ± 6,4	25	24	17	17			21 ± 4,3
43	23	20	21	30	23	20	23 ± 3,8	21	24	26	20	—	—	20 ± 3,3
45 47	77	80	79	70	77	80	77 ± 3,8	79	76	74	80			77 ± 2,7
48	1	5	3	12	8	6	6 ± 4	5	6	8	12			8 ± 3,1
50	24	21	24	28	21	18	23 ± 3,4	20	21	24	23	—	—	22 ± 1,8
52	49	44	46	40	42	44	44 ± 3,1	47	43	43	41			44 ± 2,5
54	26	30	27	20	29	32	27 ± 4,1	28	30	25	24			27 ± 2,8

LDL_1													
36	34	36	39	40	36	39	37.3 ± 2.3	38	39	40	37	35	37	37.7 ± 1.8
38	37	45	41	44	40	34	40.2 ± 4.2	37	41	40	42	36	34	38.3 ± 3.1
40	39	22	20	16	24	27	23.0 ± 4.7	25	20	20	12	29	29	22.5 ± 6.5
43	21	22	21	30	20	19	22.2 ± 4.0	21	32	28	20	24	24	24.8 ± 4.5
45	67	70	71	59	74	72	68.8 ± 5.3	68	58	60	74	62	67	64.8 ± 5.9
47	12	8	8	11	6	8	8.8 ± 2.2	11	10	12	16	14	9	12.0 ± 2.6
48	9	6	7	11	6	7	7.7 ± 2.0							
50	23	21	21	24	26	16	21.8 ± 3.4	28	34	32	29	29	30	30.3 ± 2.2
52	41	41	44	38	45	43	42.0 ± 2.5	58	49	53	46	48	48	50.3 ± 4.4
54	20	29	26	24	19	28	24.3 ± 4.1	14	17	15	21	23	22	18.7 ± 3.8
56	7	3	2	3	3	6	4.0 ± 2.0							
HDL														
36	35	29	35	35	34	36	34.0 ± 2.5	39	38	34	35	32	38	36.0 ± 2.8
38	43	42	42	41	40	45	42.2 ± 1.7	42	45	45	44	38	40	42.3 ± 2.9
40	22	29	23	24	25	19	23.7 ± 3.3	19	17	21	21	30	22	21.7 ± 4.5
43	20	20	16	26	20	13	19.2 ± 4.4	24	17	21	21	24	23	21.7 ± 2.7
45	65	65	80	62	70	82	70.7 ± 8.4	67	78	70	66	62	66	68.2 ± 5.4
47	15	15	4	12	10	5	10.2 ± 4.8	9	5	9	13	14	11	10.2 ± 3.2
48	6	8	10	14	15	6	9.8 ± 3.9	5	8	9	7	6	5	6.7 ± 1.6
50	26	22	16	26	21	13	20.7 ± 5.3	16	20	23	18	23	29	21.5 ± 4.6
52	42	43	47	38	40	46	42.7 ± 3.4	59	44	43	51	60	46	50.5 ± 7.5
54	25	27	27	22	24	35	26.7 ± 4.5	20	28	25	24	11	20	21.3 ± 5.9

*Carbon numbers 36—40, phosphatidylcholines with 34—38 acyl carbons; carbon numbers 43—47, cholesteryl esters with 16—20 acyl carbons; carbon numbers 48—56, triacylglycerols with 48—56 total acyl carbons.

TABLE VI

CARBON NUMBER DISTRIBUTION OF PHOSPHATIDYLCHOLINES OF VLDL, LDL_1, LDL, AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

Carbon number	Mole %									
	Type III					Type IV				
	1125	1224	0973	1015	Average ± S.D.	0745	0718	0738	0720	Average ± S.D.
VLDL										
32	2.0	2.9	2.5	1.2	2.1 ± 0.7	1.8	2.2	2.0	1.5	1.9 ± 0.3
33	1.3	2.0	0.5	0.7	1.1 ± 0.7	0.2	1.0	1.1	0.5	0.7 ± 0.4
34	40.4	33.6	40.7	37.6	38.1 ± 3.3	44.4	44.8	36.0	39.4	41.3 ± 4.2
36	40.0	37.7	38.8	40.4	39.2 ± 1.2	37.4	40.4	40.5	40.5	39.8 ± 1.5
38	14.9	19.2	14.5	15.6	16.1 ± 2.1	13.9	9.9	15.8	16.3	14.0 ± 2.9
40	1.4	4.6	3.0	4.5	3.4 ± 1.5	2.2	1.6	3.3	1.7	2.2 ± 0.8
LDL_1										
32	2.6	3.5	3.6	5.2	3.7 ± 0.9	2.1	2.8	1.5	2.3	2.2 ± 0.5
33	1.2	1.6	0.9	1.3	1.3 ± 0.3	0.7	1.1	0.8	1.1	0.9 ± 0.2
34	40.1	41.4	41.5	40.3	40.8 ± 0.7	42.5	44.8	34.8	40.4	40.6 ± 4.3
36	37.2	37.3	37.9	36.2	37.3 ± 0.8	37.2	40.4	41.9	39.4	39.7 ± 2.0
38	16.4	13.1	13.5	13.5	14.1 ± 1.5	14.8	9.9	16.0	14.5	13.8 ± 2.7
40	2.5	2.8	2.5	3.6	2.9 ± 0.5	2.7	1.6	3.5	2.2	2.5 ± 0.8
LDL_2										
32	2.1	3.1	2.2	1.8	2.3 ± 0.6	1.7	1.8	2.2	1.8	1.9 ± 0.2
33	1.1	1.6	0.3	1.0	1.0 ± 0.5	0.8	1.1	1.3	0.6	1.0 ± 0.3
34	41.7	38.9	38.7	39.8	39.7 ± 1.4	40.9	36.3	35.0	39.4	37.9 ± 2.7
36	38.2	37.7	40.4	37.8	38.5 ± 1.3	38.4	43.6	38.3	39.5	40.0 ± 2.8
38	13.8	16.2	15.7	15.9	15.2 ± 0.9	15.4	14.8	14.1	16.1	15.1 ± 0.9
40	3.1	3.4	2.7	4.2	3.4 ± 0.6	2.8	2.4	5.0	2.5	3.2 ± 1.2
HDL										
32	1.5	3.2	2.0	1.1	2.0 ± 0.9	1.9	1.7	1.0	1.6	1.6 ± 0.4
33	1.3	2.1	0.3	0.5	1.1 ± 0.8	0.7	1.0	0.6	0.5	0.7 ± 0.2
34	32.8	36.9	35.1	35.1	35.0 ± 1.7	43.9	38.4	31.3	41.5	38.8 ± 5.5
36	40.1	36.9	40.6	39.6	39.3 ± 1.7	38.8	43.4	41.5	38.6	40.6 ± 2.3
38	19.7	16.7	18.4	18.9	18.4 ± 1.3	12.3	14.7	20.7	14.6	15.6 ± 3.6
40	4.6	4.1	3.5	4.8	4.3 ± 0.6	2.5	0.7	4.9	3.1	2.8 ± 1.7

choline, sphingomyelin and free cholesterol molecules compared closely among the corresponding lipoprotein classes in the two types of subjects. Likewise, closely comparable are the average estimates for the sum of the cholesteryl ester and triacylglycerol molecules in the corresponding lipoprotein classes of the Type III and Type IV subjects, although there is much variation among the individual subjects in the cholesteryl ester/triacylglycerol ratios. These estimates are of the order of those obtained for the VLDL, LDL_2 and HDL particles of normolipemic subjects [9] and therefore probably represent the same complex lipoprotein interrelationships. These include the presence of less than one-half of the number of cholesteryl ester molecules in the LDL_2 compared to the VLDL particles. In many instances, however, the number of cholesteryl ester molecules in the VLDL, LDL_1 and LDL_2 particles is the same. On the basis of the data presented in Tables III and IV it is obvious that the corresponding lipoprotein particles in the Type III and Type IV subjects possess closely similar structures, although the proportions of the neutral lipid classes in the particle cores and of the polar lipid classes in the particle surface may possess significant differences.

Carbon number distribution

The distribution of the carbon numbers of the cholesteryl esters, triacylglycerols and phosphatidylcholines in the various lipoprotein fractions from the individual hyperlipoproteinemic subjects is given in Table V. It is seen that the variations among the individual subjects are of about the same order as those among the average values of different lipoprotein fractions. Furthermore, the carbon number distribution of the cholesteryl esters is about the same in all four lipoprotein classes for any one subject, although the contribution of the cholesteryl arachidonate to VLDL and LDL_1 is not readily apparent because of an overlap with the excess shorter chain triacylglycerols. The triacylglycerols of the VLDL were present in the highest amounts and allowed the most detailed assessment of their profiles. However, LDL_1 and LDL_2 fractions also contained sufficient amounts of triacylglycerols for the recognition of characteristic carbon number distributions. Clearly all of these lipoprotein fractions contained closely similar carbon number distributions, as did the HDL fraction, which contained only small amounts of triacylglycerols. We have shown elsewhere [30] that the similarities in the carbon number distribution of these triacylglycerols extend also to the fatty acid composition, but the molecular association and stereospecific distribution of the fatty acids has been determined only in the VLDL. The similarities in the carbon number distribution seen for the cholesteryl esters and triacylglycerols appear to extend to the phosphatidylcholines and sphingomyelins, which are estimated from their diacylglycerol and ceramide profiles. Since the ceramides and diacylglycerols partially overlap, the resulting carbon number profiles tend to obscure any differences among the different lipoprotein classes. Detailed analysis of the carbon numbers of the diacylglycerols and the ceramides requires a prior resolution of the two lipid classes by thin-layer chromatography in the form of the parent phospholipids or as the derived neutral lipid moieties.

Distribution of molecular species

Table VI gives the complete carbon number distribution of the diacylglycerol moieties of the phosphatidylcholines from the individual lipoprotein classes of four subjects each of the Type III and Type IV hyperlipoproteinemia. These analyses were made on capillary columns containing a non-polar liquid phase, which allowed the resolution of both carbon number and certain types of unsaturation. It is seen that the molecular species of phosphatidylcholines are closely similar in all classes of lipoproteins when isolated from the same or from different subjects. Furthermore, there are no significant differences in the distribution of the carbon number of the species between the Type III and Type IV hyperlipoproteinemia. These data are consistent with the hypothesis of an essentially complete equilibration of the molecular species of the phosphatidylcholines among the different plasma lipoprotein classes. The results of the distribution of the sphingomyelins among the VLDL, LDL_2 and HDL fractions of the Type III and Type IV subjects have already been reported [19]. The present analyses extend these findings to the LDL_1 fraction and show that its composition is clearly similar to that of the LDL_2 fraction in each of the subjects examined in both Type III and Type IV hyperlipoproteinemia (data not shown). For the present purpose it is pertinent to note that in these hyperlipoproteinemia subjects as in the normolipemic subjects analyzed previously [9] the sphingomyelin species did not fully equilibrate among the different lipoprotein classes. Specifically, both Type III and Type IV subjects possessed about 30% more of the longer chain species (C_{20}—$C_{24:1}$ acid amides) in the HDL fraction than in the VLDL fraction, with the LDL_1 and LDL_2 fractions containing intermediate proportions of the long-chain and short-chain species.

Table VII gives the fatty acid composition of the cholesteryl esters of the individual lipoprotein classes for these patients of each of the two hyperlipoproteinemia types. It is seen that the average composition of the fatty acids and therefore the molecular species of the cholesteryl esters is closely similar among the four lipoprotein classes of the Type III subjects, which compare closely to the observations made for the different lipoprotein classes of normolipemic subjects [9, 10]. In contrast to the normolipemic subjects, however, there is no evidence of an increased content of the characteristic dietary acids (oleic and palmitic) in the VLDL fraction. The cholesteryl ester composition of the different lipoprotein classes of the Type IV subjects was markedly different, with the VLDL and LDL_1 containing significantly less oleic and more linoleic acid than the HDL fraction. This suggests that these cholesteryl esters originate from different pools of fatty acids and do not equilibrate. The ratio of C_{16}/C_{18} fatty acids in the HDL fraction of the Type IV samples, however, was significantly lower than the corresponding ratio of the intact cholesteryl esters and raised the possibility that some of the linoleic acid could have been lost on storage of the samples, as the fatty acids were analyzed subsequent to the determination of the total lipid profiles. The relative content of the arachidonic acid was about the same in the cholesteryl esters of all lipoprotein classes, although a significant individual variation was also noted. In general, the molecular weight distribution of the fatty acids of the cholesteryl esters corresponded rather closely to the carbon number distribution of the cholesteryl esters given in Table V, except for VLDL and LDL_1, where the

TABLE VII

FATTY ACID COMPOSITION OF CHOLESTERYL ESTERS OF VLDL, LDL_1, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF HYPERLIPEMIC SUBJECTS

Fatty acids	Mole %							
	Type III				Type IV			
	1125	0793	1015	Average	0745	0718	0720	Average
VLDL								
14:0	1.68	2.17	1.48	1.77	1.15	1.73	1.27	1.3
16:0	16.4	19.9	14.4	16.9	16.8	15.9	16.9	16.5
16:1	3.9	3.4	3.6	6.7	4.3	5.4	4.5	4.7
18:0	1.8	1.5	1.9	1.7	2.1	1.8	1.8	1.9
18:1	25.4	26.2	19.8	23.8	22.2	27.9	26.3	27.1
18:2	46.2	33.8	5.8	43.9	44.9	41.1	45.5	43.8
18:3	0.5	1.1	0.9	0.8	0.5	2.3	0.8	1.2
20:3	0.4	0.3	0.7	0.5	0.7	1.3	0.3	0.7
20:4	3.4	2.6	4.3	3.4	2.3	2.4	2.4	2.3
LDL_1								
14:0	0.5	3.1	2.5	2.0	1.2	3.9	1.1	2.0
16:0	13.8	16.5	15.0	15.1	17.5	15.7	14.8	16.3
16:1	2.7	9.5	2.0	4.7	3.5	4.0	2.2	3.2
18:0	1.7	1.8	2.4	1.9	2.5	2.9	2.1	2.5
18:1	23.8	24.5	20.1	22.8	28.5	22.5	22.0	24.3
18:2	51.1	38.3	52.6	47.3	43.6	45.7	50.4	46.5
18:3	0.4	0.8	1.2	0.8	0.7	0.7	1.4	0.9
20:4	6.0	5.5	4.2	5.2	2.5	4.6	6.0	4.3
LDL_2								
14:0	1.2	2.5	1.8	1.8	1.8	2.5	2.5	2.2
16:0	16.0	22.8	14.9	17.8	20.0	16.0	20.4	18.8
16:1	3.8	12.6	4.3	6.9	5.0	6.1	4.5	5.2
18:0	1.5	2.0	1.4	1.6	2.1	1.6	1.5	1.7
18:1	22.6	29.3	16.2	22.7	26.1	23.9	22.4	24.1
18:2	49.2	29.9	54.4	44.1	42.3	44.0	44.7	43.6
18:3	0.6	0.4	0.9	0.6	0.4	2.3	0.8	1.2
20:3	0.5	0.3	1.0	0.6	0.6	0.8	0.4	0.6
20:4	4.3	0.9	4.9	3.4	1.5	2.6	2.5	2.2
HDL								
14:0	1.6	1.8	1.8	1.7	1.7	4.7	3.6	3.3
16:0	20.0	19.5	14.6	18.0	24.9	28.8	27.1	26.9
16:1	5.0	12.6	5.0	22.6	9.3	7.5	8.4	8.4
18:0	1.7	1.6	1.1	1.4	2.6	3.7	2.9	3.0
18:1	26.8	26.4	16.8	23.3	35.4	34.5	30.7	33.5
18:2	43.4	32.8	53.3	43.1	24.1	14.6	25.7	21.4
18:3	0.6	1.0	1.2	0.9	0.8	1.4	0.7	0.3
20:4	2.2	2.9	4.8	3.3	0.5	3.7	0.5	1.6

cholesteryl arachidonate contribution was masked by the excess triacylglycerol.

The carbon number distribution of the molecular species of the triacylglycerols of the various lipoprotein classes for each subject with Type III and Type IV hyperlipoproteinemia is given in Table V. Despite considerable individual variation, there is rather close correspondence among the average values. The best estimates are obtained for the VLDL and LDL_1 fractions, which account for a high proportion of the total lipoprotein in both Type III and Type IV hyperlipoproteinemia and which are also richest in the triacylglycerols. It is not known whether or not the similarities in the carbon number distribution extend to the composition and positional distribution of the fatty acids. We have performed [30] detailed stereospecific analyses of the triacylglycerols of VLDL on a small number of normolipemic and Type III and Type IV hyperlipemic subjects and have found certain similarities in the triacylglycerol structure. However, no comparisons have yet been made among the triacylglycerol structures of different lipoprotein classes from any normolipemic or hyperlipemic subjects.

DISCUSSION

On the basis of the detailed analysis of the lipid class and molecular species composition it is obvious that the various plasma lipoprotein fractions examined in the present study possess essentially normal composition. The accumulation of the LDL_1 fraction in both Type III and Type IV subjects appears to represent an excess of VLDL remnants (LDL_2), which possess a reasonable similarity to LDL_1 according to a variety of criteria, including the increased content of sphingomyelin. The similarities in the chemical composition of the VLDL, LDL_1, LDL_2 and HDL particles between the normolipemic and hyperlipemic subjects include the calculated particle diameters. The above findings are in accordance with certain previous studies. Stromberg et al. [27] have claimed that in Type III hyperlipoproteinemia all VLDL subfractions contain increased concentrations of total cholesterol and triacylglycerols and are relatively enriched in total cholesterol, when compared to VLDL from normolipemic subjects. The ratios of total cholesterol to triacylglycerols in the LDL subfractions of Type III hyperlipoproteinemia, however, were not significantly different from those in the LDL subfractions of the normolipemic subjects and the protein composition was also similar. Packard et al. [28] have shown that the enrichment in total cholesterol of the VLDL subfractions in Type III patients is due largely to cholesteryl esters, which are accumulated at the expense of some of the triacylglycerol. In the Type IV group, all VLDL subfractions were normal in composition. The lipid composition of the LDL subfractions of Type III and Type IV groups showed no significant deviation from that of normolipemic subjects. There were no significant differences in particle size among the Type III and Type IV patients and normal subjects of the corresponding higher density VLDL and LDL subfractions, although the lower density VLDL subfractions of the hyperlipemic subjects showed somewhat greater particle size than those of the corresponding normolipemic subjects.

On the basis of chemical analyses and computation of number of particles

Redgrave and Carlson [31] have concluded that the hyperlipidemia of Type IV subjects is accounted for by an increase in total number of VLDL and a shift towards higher particle size. Although our data cannot exclude possible changes in particle size of a minor subclass of any one lipoprotein class, the possibility of a significant deviation of the average particle size from that of normal particles is unlikely unless there has been a significant structural change in these particles, for which no evidence has yet been advanced. As a result, the well defined hyperlipoproteinemias of the Type III and Type IV, therefore, must be attributed largely to the accumulation of abnormal numbers of essentially normal lipoprotein particles, especially those of the VLDL, LDL_1 and LDL_2 density range.

Havel [32] and Hazzard and Bierman [33] have suggested that in Type III hyperlipoproteinemia there is defective conversion of VLDL into LDL with accumulation of LDL_1, while Stromberg et al. [27] have claimed that the defect may lie not only at the stage of conversion of VLDL into LDL, but also earlier in the metabolic cascade. The reason for the proposed defect in catabolism of VLDL remains uncertain. Stromberg et al. [27] have suggested that it may be related to the relative proportions of the various tetramethylurea soluble apolipoproteins, while Packard et al. [28] have considered the possibility of decreased fluidity resulting from a higher concentration of cholesteryl esters in the smaller VLDL particles as a contributing factor. In fact, the validity of the direct lipoprotein interconversion hypothesis itself must be questioned, but a satisfactory explanation has not been forthcoming.

We have recently examined the precursor—product relationship of the lipid components required for a direct conversion of VLDL into LDL and HDL postulated by Eisenberg et al. [1] and have shown that such a simple relationship does not exist in normolipemic subjects and that much more complex series of events must be involved. A similar examination of the precursor—product relationship in the present study appears to support these conclusions. The present study shows that the LDL_1 and LDL_2 fractions are closely related in their qualitative and quantitative lipid class and molecular species composition. The major differences between VLDL and LDL_1, and LDL_1 and LDL_2 are confined to the relative proportions of the triacylglycerols and cholesteryl esters. A gradual increase in the cholesteryl ester content would be anticipated if the particles were the products of VLDL degradation by lipoprotein lipase, which hydrolyses triacylglycerols but not cholesteryl esters. A simple conversion of a VLDL particle into a LDL_1 or LDL_2 particle, however, would appear to be excluded as the resulting particles contain only about one half the number of cholesteryl ester molecules present in the original VLDL. Clearly, the VLDL particle must have lost a considerable amount of cholesteryl ester along with the bulk of the triacylglycerol. Alternatively, the residual VLDL particle could have been cleaved into the LDL_1 and LDL_2 particles. Neither of these possibilities have been considered by the lipoprotein interconversion hypothesis. Neither a simple cleavage of the VLDL particle nor an indiscriminate loss of cholesteryl ester can account for the differences in the fatty acid composition of the VLDL and the LDL fractions. Since neither LDL_1 nor LDL_2 are known to support cholesteryl ester synthesis via lecithin: cholesterol acyltransferase, the differential composition of the cholesteryl

esters must have resulted from some other mechanism, such as cholesteryl ester exchange [34] or particle fusion [35], both processes having been demonstrated to occur among the plasma lipoproteins, but not included as prominent transformations in the lipoprotein interconversion hypothesis of Eisenberg et al. [1].

Just like the LDL_1 and LDL_2 of the normolipemic subjects, those of the hyperlipemic subjects also showed a marked relative increase in the sphingomyelin/phosphatidylcholine ratio, although the molecular species of neither phospholipid had undergone significant change. This change in the phospholipid class ratio indicates that the degradation of the VLDL involves in addition to the hydrolysis of the triacylglycerols also a preferential loss of phosphatidylcholine and retention of sphingomyelin in the residual particle. Since the sphingomyelin/cholesteryl ester ratio remains about the same as in VLDL, the loss of any cholesteryl ester must be accompanied by a loss of sphingomyelin, which would favour a cleavage of the VLDL particle at some stage of the degradation. It must therefore be concluded that a direct precursor—product relationship is not realized for the VLDL and LDL_1 or LDL_2 particles in the hyperlipoproteinemic patients. This also was the case in the normolipemic subjects [9, 10]. The hope that the accumulation of the presumed products would help to demonstrate the postulated precursor—product relationship was not realized, although the possibility was not entirely excluded in some modified form.

According to the lipoprotein interconversion hypothesis [1, 36, 37], plasma HDL arises largely or exclusively from the excess surface material of VLDL, which is released as an LPX-like interphase following the triacylglycerol hydrolysis. It would be anticipated that in such an instance the HDL would posses the relative proportions and composition of the sphingomyelins and phosphatidylcholines which are identical to those of VLDL. Even if allowance is made for some distortion of the molecular species of the phosphatidylcholines due to lecithin:cholesterol acyltransferase activity on HDL, which is known to favour the more unsaturated species [4], the molecular species of the sphingomyelins should have retained the composition of the precursor VLDL. The present work with the hyperlipemic plasma lipoproteins shows that the expected relationship again is not realized. Although the total sphingomyelin/phosphatidylcholine ratio in the HDL is rather close to that in the VLDL, and both lipoprotein classes possess about the same species of phosphatidylcholine, there is a marked and irreconcilable difference in the composition of the molecular species of the sphingomyelins between the VLDL and HDL fractions. As noted for normolipemic subjects the HDL contains as much as 30% more of the longer chain species of sphingomyelin than does the VLDL, even when isolated from the same subject. Other transformations than simple salvage of excess phospholipids must therefore be involved even in the VLDL—HDL conversion. We have suggested that perhaps the accumulation of the long-chain sphingomyelins in the HDL could be explained on the basis of a lateral phase separation taking place at the time of the LPX formation. Alternatively, preferential interaction of apo A_1 or apo A_2 with the long-chain sphingomyelins must be considered.

Finally, the HDL from both Type III and Type IV subjects possesses a

relatively high proportion of triacylglycerols. The lipoprotein interconversion hypothesis does not allow for the presence of triacylglycerols in the HDL fraction. Possibly significant amounts of the cholesteryl esters of HDL in the hyperlipemic subjects have been exchanged for triacylglycerols in the LDL_1, LDL_2 or VLDL, with which HDL is believed to undergo catalytic exchange via special carrier proteins [38]. Alternatively, the HDL could have been contaminated with some VLDL or LDL_1 remnants of high density, which are rich in triacylglycerols and are cleared very slowly [39]. We have discussed the above possibilities in the context of the results obtained with the lipoproteins of normolipemic subjects [9], and have proposed that particle fusion and cleavage in plasma in the native form or during ultracentrifugal isolation in the presence of the chelating agents must be advanced as a plausible mechanism for the rationalization of the differential distribution of the various lipid classes among the different lipoprotein fractions.

In view of the absence of any obvious characteristic abnormalities in the major lipid composition and structure, the accumulation of the VLDL and LDL_1 particles in Type III and Type IV hyperlipoproteinemia subjects must be sought elsewhere, e.g., in the apoprotein composition [40, 41] of these lipoproteins or in the composition and structure of their membrane receptors, which may recognize the minor differences noted.

ACKNOWLEDGEMENTS

These studies were supported by funds from the Ontario Heart Foundation, the Medical Research Council of Canada, and the National Heart, Lung and Blood Institute, NIH-NHLI-72-917, Bethesda, MD, U.S.A.

REFERENCES

1 S. Eisenberg, T. Chajek and R. Deckelbaum, Pharmacol. Res. Commun., 10 (1978) 729.
2 J.R. Patch, A.M. Gotto, Jr., T. Olivecrona and S. Eisenberg, Proc. Nat. Acad. Sci. U.S., 75 (1978) 4519.
3 A.R. Tall and D.M. Small, N. Engl. J. Med., 299 (1978) 1232.
4 J.A. Glomset, Progr. Biochem. Pharmacol., 15 (1979) 41.
5 R.J. Deckelbaum, S. Eisenberg, M. Fainaru, Y. Barckholz and T. Olivecrona, J. Biol. Chem., 254 (1979) 6079.
6 T.M. Forte, R.M. Kraus, F.T. Lindgren and A.V. Nichols, Proc. Nat. Acad. Sci. U.S., 76 (1979) 5934.
7 T. Chajek and S. Eisenberg, J. Clin. Invest., 61 (1978) 1654.
8 A.R. Tall, P.H.R. Green, R.M. Glickman and J.W. Riley, J. Clin. Invest., 64 (1979) 977.
9 A. Kuksis, J.J. Myher, K. Geher, W.C. Breckenridge, G.J.L. Jones and J.A. Little, J. Chromatogr., 224 (1981) 1.
10 A. Kuksis, J.J. Myher, K. Geher, J. Shepherd, C.J. Packard, J.D. Morrisset, O.D. Taunton and A.M. Gotto, Jr., Atherosclerosis, 41 (1982) 221.
11 Anon., Manual of Laboratory Operations. Lipid Research Clinics Program. Vol. 1. Lipid and Lipoprotein Analysis, National Heart and Lung Institute, National Institutes of Health, Bethesda, MD, 1974, DHEW Publication No. (NIH) 75-628.
12 F.T. Hatch and R.S. Lees, Advan. Lipid Res., 6 (1968) 1.
13 G.F. Maguire and W.C. Breckenridge, Clin. Biochem., 8 (1975) 161.
14 O.H. Lowry, N.J. Rosebrough, A.K. Farr and R.J. Randall, J. Biol. Chem., 193 (1951) 265.

15 A. Kuksis, J.J. Myher, K. Geher, A.G.D. Hoffman, W.C. Breckenridge, G.J.L. Jones and J.A. Little, J. Chromatogr., 146 (1978) 393.
16 A. Kuksis, J.J. Myher, L. L. Marai and K. Geher, J. Chromatogr. Sci., 13 (1975) 423.
17 J.J. Myher, A. Kuksis, L. Marai and S.K.F. Yeung, Anal. Chem., 50 (1978) 557.
18 A. Kuksis, J.J. Myher, W.C. Breckenridge and J.A. Little, in K. Lippel (Editor), Report of the High Density Lipoprotein Methodology Workshop, NIH Publication No. 79-1661, Bethesda, MD, 1979, pp. 142—163.
19 J.J. Myher, A. Kuksis, W.C. Breckenridge and J.A. Little, Can. J. Biochem., 59 (1981) 626.
20 A. Kuksis, Fette Seifen Anstrichm., 73 (1971) 130.
21 A. Kuksis, J.J. Myher, K. Geher, N.A. Shaikh, W.C. Breckenridge, G.J.L. Jones and J.A. Little, J. Chromatogr., 182 (1980) 1.
22 A. Kuksis, W.C. Breckenridge, J.J. Myher and G. Kakis, Can. J. Biochem., 56 (1978) 630.
23 T. Sata, R.J. Havel and A.L. Jones, J. Lipid Res., 13 (1972) 757.
24 S. Eisenberg and R.I. Levy, Advan. Lipid Res., 13 (1975) 1.
25 V.P. Skipski, in G.J. Nelson (Editor), Blood Lipids and Lipoproteins, Wiley-Interscience, New York, 1972, pp. 471—583.
26 D.M. Lee, in C.E. Day and R.S. Levy (Editors), Low Density Lipoproteins, Plenum Press, New York, 1976, pp. 1—74.
27 P. Stromberg, D. Ballantyne, F.C. Ballantyne, J.L.H.C. Third and D.K. Bedford, Clin. Chim. Acta, 79 (1977) 163.
28 C.J. Packard, J. Shepherd, S. Toerns, A.M. Gotto, Jr. and O.D. Taunton, Biochim. Biophys. Acta, 572 (1979) 269.
29 J.L. Witztum, M.A. Dillingham, W. Giese, J. Bateman, C. Diekman, E. Kammeyer, B. Laufuss, S. Weidman and G. Schonfeld, N. Engl. J. Med., 303 (1980) 907.
30 J.J. Myher, A. Kuksis, W.C. Breckenridge and J.A. Little, J. Amer. Oil Chem. Soc., 57 (1980) Abstr. No. 413.
31 T.G. Redgrave and L.A. Carlson, J. Lipid Res., 20 (1979) 217.
32 R.J. Havel, Ann. N.Y. Acad. Sci., (1980) 16.
33 W.R. Hazzard and E.L. Bierman, Metabolism, 24 (1975) 817.
34 J. Ihm, J.A.K. Harmony, J. Ellsworth and R.L. Jackson, Biochem. Biophys. Res. Commun., 93 (1980) 1114.
35 T.E. Grow and M. Fried, J. Biol. Chem., 253 (1978) 8034.
36 S. Eisenberg, Ann. N.Y. Acad. Sci., (1980) 30.
37 A.R. Tall and D.M. Small, Advan. Lipid Res., 17 (1980) 1.
38 Y.L. Marcel, C. Vezina, B. Teng and A. Sniderman, Atherosclerosis, 35 (1980) 127.
39 T.A. Musliner, P.N. Herbert and M.J. Kingston, Biochim. Biophys. Acta, 575 (1979) 277.
40 G. Utermann, G. Albrecht and A. Steinmetz, Clin. Gen., 14 (1978) 351.
41 R.E. Gregg, L.A. Zech, E.J. Schaefer and H.B. Brewer, Jr., Science, 211 (1981) 584.