Journal of Chromatography, 224 (1981) 1—23
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 835

LIPID CLASS AND MOLECULAR SPECIES INTERRELATIONSHIPS AMONG PLASMA LIPOPROTEINS OF NORMOLIPEMIC SUBJECTS

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(First received October 1st, 1980; revised manuscript received January 4th, 1981)

SUMMARY

As evidence of lipoprotein interconversion and/or equilibration, a gas—liquid chromatographic (GLC) examination was made of the lipid class and molecular species interrelationships among the major fasting plasma lipoprotein fractions within each of seven male and four female normolipemic 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 GLC of the intact cholesterol and glycerol esters and of ceramides. In general, each lipoprotein fraction possessed a well defined lipid class composition, which was characterized by a dramatically decreasing triacylglycerol and an increasing phospholipid and cholesteryl ester content when progressing from the very low (VLDL), to the low (LDL_2) and high (HDL) density lipoproteins, as already established by conventional analyses. However, the LDL_2 contained about a two times higher proportion of total phospholipids as sphingomyelin than VLDL and HDL. Furthermore, the sphingomyelins of the HDL fraction contained about 30% more of the higher molecular weight species than the sphingomyelins of either VLDL or LDL. Smaller differences were seen in the molecular species composition of the phosphatidylcholines, cholesteryl esters and triacylglycerols among the corresponding fractions of lipoproteins. In general, the lipid class and molecular species distribution is incompatible with the hypothesis which postulates VLDL conversion into LDL and HDL under the influence of lipoprotein lipase and lecithin:cholesterol acyltransferase. The significant differences noted in the lipid class and molecular species distribution suggest that the true transformations of the lipoproteins are much more complex and may also involve cholesteryl ester-triacylglycerol, triacylglycerol and phosphatidylcholine exchanges via appropriate carrier plasma proteins, as well as possible phase separation of lipids during the removal of the excess surface material from the VLDL remnants, as already demonstrated in in vitro experiments. It is concluded that a direct GLC analysis of the neutral and polar lipid components of plasma lipoprotein classes provides important evidence

of lipoprotein interrelationships which may be utilized to test existing and new hypotheses of lipoprotein interconversion.

INTRODUCTION

Extensive recent work [1,2] has established that the protein moiety of human plasma very low density lipoprotein (VLDL) is a precursor of the protein moiety of low density lipoprotein (LDL). In normal humans, all of the apoprotein B moiety of LDL may be derived from VLDL and all of the VLDL apoprotein B may be converted to LDL-apoprotein B. Detailed analyses of VLDL and LDL particles have revealed that they contain approximately the same number of apoprotein B molecules in single lipoprotein [3,4] implying a direct particle interconversion. The interconversion of VLDL into LDL is mediated by lipoprotein lipase and it results in removal of most of the triacylglycerol, as much as 90% of the phosphatidylcholine and 60% of the sphingomyelin, while the cholesteryl esters are largely retained in the newly formed LDL [3]. Since much of the unhydrolyzed VLDL phosphatidylcholine and sphingomyelin is found in the high density lipoprotein (HDL) density range, Eisenberg [5] has suggested that during the degradation of human VLDL to LDL there may be HDL formed. A similar conclusion had been reached earlier on theoretical grounds by Schumaker and Adams [6], who also proposed that this transformation is mediated by lecithin:cholesterol acyltransferase. Recently several laboratories [7—10] have advanced more or less detailed models for these interconversions, including the above enzymatic pathways. Unlike previous proposals of general cascading transformations of lipoproteins, the latter models are sufficiently specific for analytical testing. The postulated largely self-contained processes [11,12] dictate defined interrelationships among the various components of the precursor and product particles, including the lipids of their polar surfaces and of neutral cores [13,14].

In the following study we have examined the precursor—product relationships among the major plasma lipoproteins by comparing the mass distribution of the lipid classes and molecular species in individual samples of plasma from a significant number of fasting normolipemic subjects. While the data so obtained are consistent with the basic hypothesis of VLDL conversion into LDL and HDL, significant differences are also seen, which suggest a more complex series of transformations than those previously proposed.

MATERIALS AND METHODS

Blood samples were obtained in the fasting state (12—14 h) from eleven healthy normolipemic subjects: seven males, 25—55 years old, and four females, 26—63 years old. The subjects lived at home and subsisted on diets of free choice.

Isolation and characterization of lipoproteins

Lipoproteins were isolated essentially according to the procedure described by Hatch and Lees [15]. Briefly, 5-ml aliquots of plasma were placed in 6.5-

ml cellulose nitrate tubes and overlayered with saline [density (d) = 1.006 g/ml] containing 1% Na_2EDTA. They were centrifuged at 10°C at 100,000 g for 18 h. The supernatant fraction (VLDL) and infranatant fraction were recovered by tube slicing. The infranatant fraction was made up to 5 ml in a volumetric flask at 20°C with saline and then adjusted to d = 1.019 g/ml by the addition of anhydrous solid potassium bromide. The material was placed in a centrifuge tube overlayered with saline adjusted to d = 1.019 g/ml and centrifuged at 100,000 g for 20 h. The supernatant fraction (LDL_1) was not investigated further. The infranatant was reconstituted to 5 ml with saline adjusted to d = 1.019 g/ml. The potassium bromide addition was continued to obtain supernatant fractions of d = 1.063 g/ml (100,000 g, 24 h) (LDL_2) and d = 1.21 g/ml (110,000 g, 48 h) (HDL). A random check of the procedure of density adjustment by picnometry indicated that the adjustments were within 2—3% of the projected value. Each supernatant fraction was washed once under conditions of isolation and collected in a final volume of 2 ml, which was dialyzed against saline. Each fraction was checked for electrophoretic mobility on agarose gel electrophoresis [16]. The apoprotein make-up was assessed by solubility measurements in tetramethylurea [17]. Fractions were also checked by double immunodiffusion against rabbit anti-human albumin, antihuman LDL and HDL. On the basis of agarose gel electrophoresis, the VLDL preparations possessed pre-β mobility with no evidence of chylomicron material or trail to the origin. The LDL_2 preparations showed only proteins of β-mobility. The HDL fraction had α-mobility. Occasionally small amounts of sinking pre-β lipoprotein representing less than 5% of optical density of the total HDL fraction were found. A double immunodiffusion showed the expected cross-precipitation bands only. The protein concentration in each lipoprotein fraction was determined by the method of Lowry et al. [18] using bovine serum albumin as a standard. Preparations of VLDL were extracted with diethyl ether after colour development. In some instances the protein concentration was determined using the modification of the method of Lowry et al. [18] described by Sata et al. [19].

Dephosphorylation and isolation of lipids

Portions of the solutions of the various density fractions (equivalent to 0.1—0.2 ml of plasma) were placed into 18-ml centrifuge tubes with screw caps (PTFE-lined). The contents were diluted with 1 ml of water, and 1 ml of diethyl ether was added followed by 2 ml of a solution (0.1 mg/ml of Tris buffer, pH 7.3) of phospholipase C (α-toxin of *Clostridium welchii*, Sigma, St. Louis, MO, U.S.A.). Finally 1.3 ml of 10% calcium chloride solution were added and the solution mixed. The incubation was performed at 33°C for 2 h with shaking. The enzyme digest was partitioned once with 10 ml chloroform—methanol (2:1) containing 200 μg of tridecanoylglycerol, and the solution centrifuged for 10 min at low speed to break any emulsion. The lower chloroform phase was collected and passed through a Pasteur pipet containing 2 cm length of anhydrous sodium sulfate and the filtrate was evaporated to dryness under nitrogen. To the dry residue were added 75 μl of TRISIL-BSA (Pierce, Rockford, IL, U.S.A.) and the vial sealed with a screw cap. After 30 min at room temperature, the silylation mixture was transferred to a conical sampling

vial and the vial sealed with a clamp-on silicone septum and transferred to a sampling table for gas chromatography.

Gas—liquid chromatography

The quantitative lipid profiles of the various lipoprotein classes were determined by means of an automated gas—liquid chromatography (GLC) system equipped with an automatic liquid injector, programmed heating, cooling and equilibration cycles, and an electronic peak area integrator. The separations were accomplished on a 50 cm × 0.2 cm I.D. stainless-steel column packed with 3% OV-1 (a methyl silicone 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 peak identification and composition of samples were calculated 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 [20,21]. Fatty acid methyl esters of the various lipid classes were prepared by transmethylation with sulfuric acid—methanol and the methyl esters separated, identified and quantitated by GLC on 10% EGSS-X (an ethyleneglycolsuccinate—silicone copolymer), as previously described [22]. The carbon numbers of isolated diacylglycerols [23] and ceramides [24] were determined by GLC as previously described. For this purpose the diacylglycerols and ceramides were first converted into the *tert.*-butyldimethylsilyl ethers. The GLC analyses were performed on a Beckman GC-4 gas chromatograph equipped with a stainless-steel column (50 cm × 0.2 cm I.D.), containing 3% OV-1 packing.

Calculations

In addition to the automated corrections of peak areas for differences in flame ionization response and recovery, the following additional calculations were performed on the peak areas following the completion of the automated GLC. Due to a partial overlap of the higher molecular weight diacylglycerols, ceramides and the lower molecular weight cholesteryl esters in the C_{40}—C_{42} range of carbon numbers the content of phosphatidylcholine of the lipoprotein fractions was calculated from the peak areas of carbon number C_{36}—C_{38} and that of sphingomyelin from the peak areas of carbon number C_{34}, as previously described [25]. Total phosphatidylcholine = total DG × 1.28, where total DG = corrected $(C_{36} + C_{38})/0.81$, and corrected $C_{36} + C_{38} = (C_{36} + C_{38}) - kC_{34}$ ceramide; and where C_{34} ceramide = $C_{34} - 0.051 \times C_{36}$, and $k = 1$ for HDL and VLDL, and 1/2 for LDL. The total sphingomyelin = total ceramide × 1.28, where total ceramide = C_{34} ceramide × 0.758/0.30 for VLDL and LDL, and C_{34} ceramide × 0.758/0.25 for HDL. The factor 0.758 is the ratio of the response factors for ceramide and diacylglycerol TMS ether (0.758 = 0.681/0.898). The factors 0.81 and 0.30 represent the proportion of the measured diacylglycerol and ceramide species of plasma phosphatidylcholine and sphingomyelin as derived from analyses of large plasma pools. The multiplication factor 1.28 converts the ceramides and diacylglycerols into the corresponding phosphorylcholine derivatives.

The core radii of the lipoprotein particles were calculated on the basis of

the surface to volume ratio of a sphere as previously described [24,26]. Surface area = $4\pi r^2 = k[Apl(PL) + Achol\ (C)]$ and volume of core equals $4/3\pi^3 = k[Vtg(TG) + Vce)CE)]$, where k is a proportionality constant dependent on the number of particles per unit lipid mass; *PL*, *C*, *TG*, and *CE* are the mole percentages of phospholipid, free cholesterol, triacylglycerol, and cholesteryl ester, respectively; $Apl = 0.685\ nm^2$ and $Achol = 0.391\ nm^2$ are the molecular surface areas of phospholipid and free cholesterol, respectively. $Vtg = 1.556\ nm^3$ and $Vce = 1.068\ nm^3$ are the molecular volumes of triacylglycerol and cholesteryl ester, respectively. The total radii of the particles are obtained by adding the thickness of the surface monolayer (2.0 nm) to the core radii [19]. The number of neutral lipid molecules in the particle cores is 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 is calculated by dividing the appropriate proportion of the total area by the area of the average phospholipid and free cholesterol molecule, respectively [27].

RESULTS

Total lipid profiles

The total lipid profiles of the VLDL, LDL_2 and HDL fractions from a normolipemic male subject in the fasting state are shown in Fig. 1. In this elution pattern the various lipid subclasses are represented by their total acyl, acyl plus sterol, or acyl plus sphingosine carbon numbers. Peak 30 represents the internal standard, tridecanoylglycerol, which has been added in equal amounts

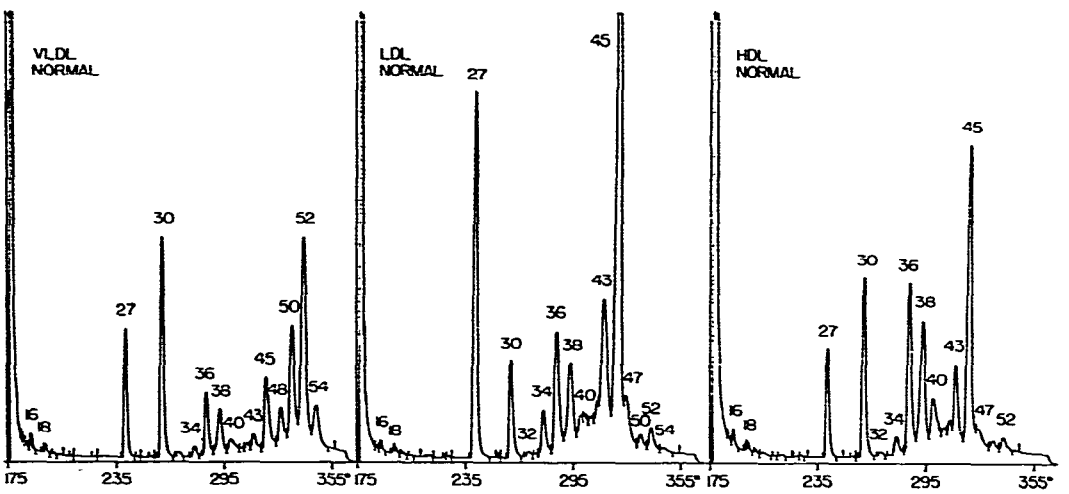

Fig. 1. GLC profiles of total lipids of plasma lipoproteins of fasting normolipemic subjects. Conditions of high-temperature GLC as given in text. Peaks: 16 and 18, trimethylsilyl esters of free fatty acids with 16 and 18 acyl carbons; 27, trimethylsilyl ether of cholesterol; 30, tridecanoylglycerol, 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 a total number of 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.

to each of the lipoprotein samples. The relatively small VLDL fraction is characterized by the presence of a high proportion of triacylglycerols (peaks 48—54) and smaller amounts of cholesteryl esters (peaks 43—47), which are resolved according to their carbon numbers, and free cholesterol (peak 27). A carbon number resolution is also seen for the major diacylglycerols (peaks 36—42) derived from the plasma phosphatidylcholines, while only one distinct carbon number (peak 34) is seen for the ceramides derived from sphingomyelin (the other peaks are partially hidden under the diacylglycerol peaks). The LDL_2 lipid profile is characterized by a high proportion of cholesteryl ester and smaller amounts of phospholipid and free cholesterol, and a very small proportion of triacylglycerols. The proportion of the triacylglycerols is reduced still further in the HDL fraction, which contains a high proportion of phospholipid and cholesteryl esters, with smaller amounts of free cholesterol. It should also be noted that the lipid profiles differ in the relative proportions of the peaks for free cholesterol and for cholesteryl esters, and in the ratios of the peaks representing sphingomyelin (peak 34) and phosphatidylcholines (peaks 36—42). Essentially identical elution patterns were recorded for the corresponding

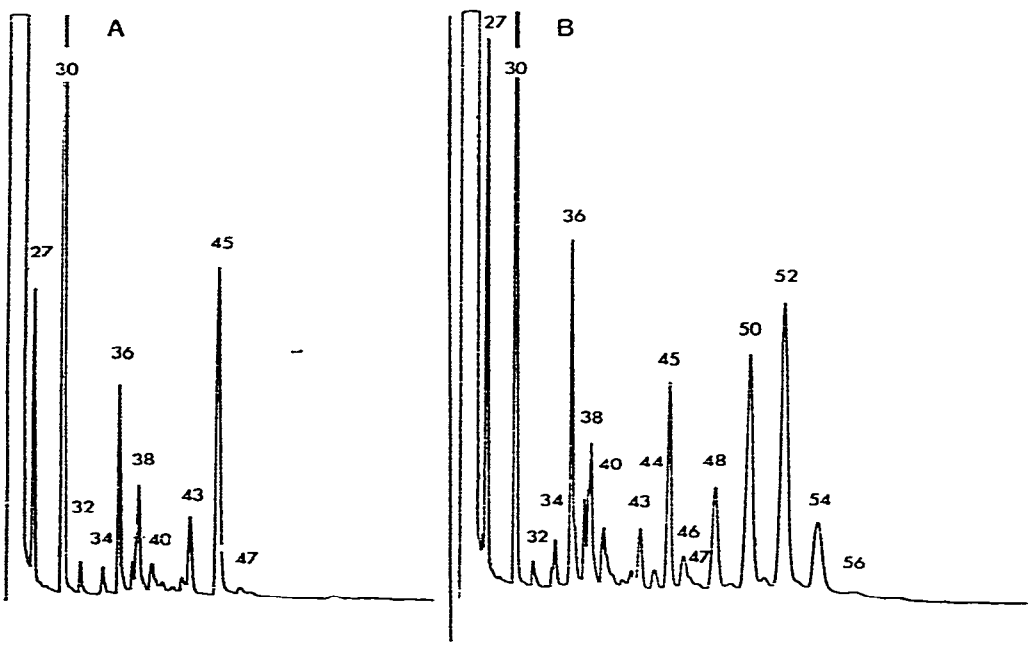

Fig. 2. Capillary GLC profiles of total lipids of plasma lipoproteins of fasting normolipemic subjects. (A) An intermediate density lipoprotein fraction containing negligible amounts of triacylglycerols; (B) VLDL. Peaks 44 and 46 represent triacylglycerols with 44 and 46 acyl carbons; other peaks as in Fig. 1. Capillary GLC conditions: a glass capillary column (5 m × 0.25 mm I.D.) coated with SP-2100 (Supelco, Bellefonte, PA, U.S.A.) and installed in a Hewlett-Packard Model 5880A gas chromatograph equipped with a splitless injector and a hydrogen flame ionization detector. The injector was maintained at 320°C, and the detector at 340°C. The hydrogen carrier gas-flow was maintained with a head pressure of 8 p.s.i. Temperature program: 240°C isothermal for 1 min, followed by temperature programming at 10°C/min to 295°C, then at 3.5°C/min to 340°C. Peak 54 was eluted in 15.80 min at 328°C. Column bleed was minimal and was automatically subtracted via the single column compensation mode of the microprocessor terminal.

lipoprotein classes from other normal subjects on the free choice diets. In VLDL, the low molecular weight triacylglycerols overlap with the cholesteryl esters, as shown by capillary GLC (Fig. 2).

Quantitative composition

The weight percentages of protein and lipid in the VLDL, LDL_2 and HDL fractions isolated from the normal subjects are given in Table I. The values were obtained by summing the estimates for individual lipid classes, calculated from the total lipid profiles, and the estimates for total protein measured independently on aliquots of the lipoprotein solutions. It is seen that the percentage of protein in the VLDL from both sexes averages 9±1%. This corresponds favourably with an average value of 8% reported by Skipski [28]. Eisenberg and Levy [4] have tabulated weight percentages for protein ranging from 4—11% for various subfractions of VLDL depending on particle size. The percentage of protein in the LDL_2 fraction from these subjects averaged 20±1%, which also corresponds well with the average content of protein in this lipoprotein class as tabulated by Skipski [28] and by Lee [29]. The weight percentage of protein in the HDL fraction of these subjects was 50±3%, which could have represented a 1:4 mixture of HDL_2 and HDL_3. Shen et al. [30] have reported 41 and 55% protein for HDL_2, and HDL_3, respectively, of normal human plasma, while Hatch and Lees [15] have estimated the ratio of these two lipoproteins to be about one part HDL_2 and four parts HDL_3. These results would suggest that the normolipemic subjects selected for this study possess the normal lipid:protein ratios for these lipoprotein particles, as well as demonstrate that the GLC quantitation of the lipids corresponded closely with the independently measured protein values. Table I also gives the weight per cent composition of the major lipid classes as measured by GLC. The VLDL is seen to contain 18—22% total phospholipid (phosphatidylcholine plus sphingomyelin), 57—65% triacylglycerol, 11—17% cholesteryl ester and 5—7% free cholesterol. These estimates are in good agreement with the values tabulated for VLDL by Skipski [28]. The total phospholipid content of the LDL_2 fraction is seen to range from 28—34%, the cholesteryl ester content from 46—53% and free cholesterol from 10—12%, with the triacylglycerols accounting for only 6—8% of the total lipid. Again the lipid class proportions are very consistent among the different subjects and well within the range of normal values reported in the literature [28,29]. Likewise, the HDL fraction isolated and analyzed in this study possesses essentially normal composition, with the total phospholipids ranging from 48—56%, the cholesteryl esters from 35—41% and triacylglycerols and free cholesterol from 4—7% of the total lipid. These values again are in excellent agreement with the values reported by Skipski [28].

Lipid class ratios

The molar ratios of the various lipid classes in the major lipoprotein fractions of each normolipemic individual are given in Table II. These ratios are extremely consistent and show only marginal differences between the normolipemic subjects of the two sexes, as well as among different individuals of the same sex. The average ratios for the major lipid classes in the VLDL fraction corre-

TABLE I

OVERALL COMPOSITION OF VLDL, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF NORMOLIPEMIC SUBJECTS

Individual lipid classes estimated as % of total lipid. Each estimate is an average of two or three replicate gas chromatographic analyses.

Chemical component	Males								Females				
	1	2	1690	1765	1767	1689	1858	Average	3	4	18	19	Average
						Weight (%)							
VLDL													
Total protein	10	11	8	8	8	9	9	9±1	10	9	8	9	9±1
Total lipid	90	89	92	92	92	91	91	91±1	90	91	92	91	91±1
PC*	20	18	16	19	18	18	16	18±1	18	19	16	19	18±1
SPH	2	2	2	2	2	2	2	2±0	2	2	2	2	2±0
CE	15	14	11	13	12	14	12	13±1	16	17	12	14	15±2
TG	57	61	65	58	61	59	65	61±3	58	56	65	59	60±4
FC	7	5	5	7	6	7	6	6±1	5	6	5	6	6±1
LDL_2													
Total protein	21	21	20	20	20	20		20±1	19	21	22	19	20±1
Total lipid	79	79	80	80	80	80		80±1	81	79	78	81	80±1
PC	25	25	23	22	23	26		24±1	24	27	25	23	25±2
SPH	7	9	6	6	9	9		8±1	9	7	8	9	8±1
CE	51	46	51	53	49	49		50±2	49	47	48	50	49±1
TG	7	8	8	8	8	6		8±1	6	7	7	7	7±1
FG	10	11	12	11	12	11		11±1	12	11	11	12	12±1
HDL													
Total protein	54	50	57			53	51	53±3	51	56	53	52	53±2
Total lipid	46	50	43			47	49	47±3	49	44	47	48	47±2
PC	44	48	43			43	45	44±3	47	49	45	44	46±2
SPH	5	8	7			5	5	6±1	6	6	5	6	6±0
CE	40	36	39			38	40	39±2	35	35	39	41	38±3
TG	4	7	5			5	4	5±1	6	6	5	4	5±1
FC	7	7	6			9	6	7±1	5	5	6	6	6±1

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

TABLE II

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

Each ratio is an average of two or three estimates from replicate gas chromatographic analyses.

Chemical components	Males								Females				
	1	2	1690	1765	1767	1689	1858	Average	3	4	18	19	Average
						Mole/Mole							
VLDL													
FC/TC*	0.43	0.37	0.44	0.48	0.47	0.44	0.45	0.44	0.34	0.36	0.39	0.40	0.37
FC/PL	0.63	0.47	0.59	0.67	0.64	0.66	0.67	0.62	0.51	0.54	0.57	0.54	0.54
SPH/PC	0.13	0.12	0.14	0.14	0.11	0.14	0.11	0.13	0.13	0.09	0.13	0.12	0.12
EC/TG	0.35	0.30	0.23	0.30	0.25	0.33	0.24	0.29	0.36	0.39	0.25	0.31	0.33
FC/SPH	5.6	4.3	4.8	5.4	6.3	5.3	6.6	5.5	4.3	7.3	5.0	4.6	5.3
FC/PC	0.71	0.52	0.67	0.77	0.71	0.75	0.75	0.70	0.58	0.60	0.63	0.57	0.60
LDL_2													
FC/TC	0.25	0.29	0.28	0.26	0.28	0.27		0.27	0.30	0.28	0.29	0.28	0.29
FC/PL	0.66	0.69	0.80	0.80	0.73	0.62		0.72	0.72	0.67	0.71	0.71	0.70
SPH/PC	0.30	0.37	0.31	0.28	0.38	0.36		0.33	0.30	0.28	0.36	0.28	0.31
EC/TG	10.2	7.3	9.5	6.4	6.5	7.3		7.9	10.1	9.1	9.8	9.5	9.6
FC/SPH	2.8	2.5	3.3	3.6	2.6	2.4		2.9	2.6	2.8	2.7	2.5	2.7
FC/PC	0.84	0.94	1.04	1.02	1.02	0.83		0.95	1.00	0.86	0.97	0.98	0.95
HDL													
FC/TC	0.20	0.23	0.19			0.17	0.22	0.20	0.19	0.20	0.19	0.17	0.19
FC/PL	0.27	0.23	0.23			0.27	0.29	0.26	0.21	0.20	0.23	0.23	0.23
SPH/PC	0.12	0.16	0.18			0.13	0.11	0.14	0.12	0.13	0.12	0.14	0.13
EC/TG	6.26	10.0	7.5			10.2	9.6	8.7	7.5	7.1	7.7	8.4	7.7
FC/SPH	3.3	2.4	1.6			2.5	3.0	2.6	2.0	1.6	1.9	2.0	1.9
FC/PC	0.29	0.25	0.28			0.31	0.34	0.29	0.23	0.21	0.23	0.26	0.23

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

spond closely to the values reported in the literature [19,27,31], or to the values that can be calculated from published data [28]. The FC/TC ratio for VLDL averages about 0.4, which is somewhat lower than that reported by Eisenberg [27]. This free cholesterol occurs in a ratio of just less than one molecule cholesterol per two molecules of total phospholipid (0.5—0.6), which has been claimed [32] to be a stable cholesterol—phospholipid complex in artificial liposomes (no sterol—sterol contacts). These VLDL particles possess an extremely consistent SPH/PC ratio (0.12—0.13), the significance of which is not known. Relatively constant is also the ratio of the two neutral ester classes of the VLDL core (EC/TG = 0.29—0.33).

The lipid class ratios of LDL_2 are likewise very constant and again of the order that can be calculated from published data [28,33]. The FC/TC ratio in LDL_2 is significantly lower (0.28) than that in VLDL (0.40) because of the large amounts of cholesteryl ester in the neutral lipid core. The SPH/PC ratio (0.31—0.33) is about two times higher in LDL_2 than in VLDL. The FC/PL ratio in LDL_2 is significantly higher (0.70) than that in VLDL (0.60) and suggests some cholesterol—cholesterol contacts [32].

The HDL particles had the lowest variation in lipid class ratios of the three lipoprotein classes. The average values obtained for HDL in the present study again are in good agreement with those calculated from data reported in the literature [28,34]. The HDL fractions had a slightly lower FC/TC ratio (0.20) than the LDL_2 fractions (0.27), while the FC/PL ratios of HDL (0.23—0.26) were only one half those in VLDL (0.60) and one third those in LDL_2 (0.70). The HDL and VLDL possessed essentially identical SPH/PC ratios, but the sphingomyelins in the two instances were made up of distinctly different molecular species (see below).

Particle size distribution

The calculated particle size distribution for the VLDL, LDL_2 and HDL fractions for each subject is given in Table III. For this calculation the lipid core model was assumed to be valid for all lipoproteins and all free cholesterol, phosphatidylcholine and sphingomyelin were assigned to the polar surface shell, while all the triacylglycerol and cholesteryl ester were assigned to the neutral lipid core of the sphere. The calculated core radii for the VLDL, LDL_2 and HDL averaged 168, 74 and 45 Å, in males respectively. Except for HDL, the equivalent particle radii (core radii plus 20 Å surface shell) are within the reach of the sizes characteristic of normolipemic subjects obtained previously either by calculation from results of conventional analyses or by direct measurement in the electron microscope [30]. Kezdy [35] has reported particle radii of 200 and 95.9 Å for VLDL and LDL, respectively. The core radii of the HDL particles obtained in the present study are about twice that (19 Å) measured by Shen et al. [30] for HDL_3 and about one and a half times that (30 Å) measured by Kezdy [35] for HDL_2. However, the core radii calculated by the present method using the lipid composition of Shen et al. [30] and Kezdy [35] gave values of 39 Å and 50 Å for the HDL_3 and HDL_2 particles, respectively, which is in excellent agreement with the results of this work. The particle size calculated for HDL from the GLC data is overestimated by about 10%. This is due to the failure of the GLC method [25] to account for the

presence of lysophosphatidylcholine and the serine, inositol and ethanolamine phosphatides, which make up a total of 10% of the polar lipids of the HDL surface shell [34]. However, the fact that the calculated particle size of HDL is always larger than the measured size indicates some significant deviation from the ideal spherical model proposed. Furthermore, the triacylglycerol core is known to dissolve about 5 mole % free cholesterol [36], hence the present method of calculation may underestimate the true particle size of the VLDL and LDL_2 by a small percentage because all the free cholesterol in the particle has been assigned to the surface shell. The particle size of the VLDL is seen to vary more than that of the LDL_2 which varies more than that of HDL. These results are consistent with certain previous reports in the literature [30,34], which also have noted a greater individual variability in the VLDL and LDL than in HDL particle size among normolipemic individuals. Table III also includes the calculated masses of the various lipoprotein particles. The masses have been expressed in daltons assuming a molecular weight of 10,000 for the apoprotein moieties [27]. The calculated mass of the HDL particles is about one half that of the LDL_2 particles, which is consistent with the results of other workers [27,29,30].

Surface and core composition

The calculated concentrations of the lipids at the surface and in the core of the VLDL, LDL_2 and HDL particles as the number of molecules are given in Table IV. The average number of molecules calculated for the surface and core of the VLDL and LDL_2 particles is of the order reported by Kezdy [35] for particles of comparable radii. There is, however, considerable individual variation, which reflects the variability in the percent lipid composition of the individual samples pointed out above. The numbers of the various molecules calculated for the surface and the core of the HDL particles greatly exceed the numbers required for the dimensions of these particles measured in the electron microscope or derived from flotation data [30]. However, the estimated number of the molecules in the surface shell and in the core of the HDL particles is consistent with the numbers calculated from the compositional data utilized by Shen et al. [30] and Kezdy [35]. Table IV allows a comparison of the number of molecules of specific lipid classes in the various presumptive precursor and product molecules. Of special interest are the comparisons of the number of cholesteryl ester molecules in the corresponding VLDL and LDL_2 particles. On the basis of the average values it would appear that the LDL_2 particles contain less than one half of the number of cholesteryl ester molecules found in the VLDL, although in specific instances the numbers of these molecules may be identical in the presumed precursor and product particles. In other cases the LDL_2 particles are seen to contain only about a quarter of the number of cholesteryl ester molecules in the precursor VLDL, which suggests cleavage of the VLDL particles during the digestion of the triacylglycerol by lipoprotein lipase. If cleavage of the VLDL particles occurs, the excess cholesteryl ester may be lost as a complex with apoprotein E [37]. In any event, the ratio of sphingomyelin to cholesteryl ester remains the same in both VLDL and LDL_2. A comparable ratio of sphingomyelin and

TABLE III

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

Each value is an average of two or three estimates from replicate gas chromatographic analyses.

Parameter	Males								Females				
	1	2	1690	1765	1767	1689	1858	Average	3	4	18	19	Average
VLDL													
Core radius*	141	177	191	148	162	160	194	168	178	161	205	158	175
Particle weight**	10.4	19.4	23.7	11.7	15.1	14.6	24.9	17.1	19.5	14.9	28.9	14.1	19.3
LDL_2													
Core radius	75	67	78	86	71	66		74	66	64	67	69	67
Particle weight	2.3	1.7	2.6	3.2	2.0	1.7		2.3	1.6	1.6	1.7	1.8	1.7
HDL													
Core radius	45	42	46			42	44	45	41	39	46	45	45
Particle weight	1.0	0.9	1.1			0.9	1.0	1.0	0.9	0.8	1.2	1.0	1.0

*Particle radius (A) = core radius (A) plus thickness of outer shell (20 A).

**Particle weight in daltons $\times 10^{-6}$.

TABLE IV

CALCULATED CONCENTRATION OF LIPIDS AT THE SURFACE AND IN THE CORE OF VLDL, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF NORMOLIPEMIC SUBJECTS (NUMBER OF MOLECULES)

Each value is an average of two to three estimates from replicate gas chromatographic analyses. Abbreviations as in Table I.

Chemical components	Males								Females				
	1	2	1690	1765	1767	1689	1858	Average	3	4	18	19	Average
Surface components													
VLDL													
PC	2376	4021	4348	2500	3143	2959	4466	3402	3917	3316	5127	3134	3873
SPH	316	519	641	374	373	440	531	456	553	287	687	407	483
FC	1721	2150	2996	1963	2296	2268	3435	2158	2310	2026	3344	1830	2377
LDL_2													
PC	579	429	584	709	464	431		532	398	419	419	437	418
SPH	183	167	191	211	191	58		183	161	133	157	184	159
FC	496	410	624	741	485	367		520	403	369	414	442	407
HDL													
PC	284	261	303			242	277	273	244	219	301	288	263
SPH	38	28	42			35	38	36	28	29	41	39	34
FC	87	68	80			102	80	83	56	46	80	77	66
Core components													
VLDL													
TG	6107	12447	16249	7207	9760	8988	17045	11114	11973	8825	19842	8775	12352
CE	2220	3729	3696	2106	2519	2957	4134	3051	4541	3532	4965	2117	3939
LDL_2													
TG	143	134	185	244	139	85		155	94	98	101	116	102
CE	1478	998	1629	2100	1217	1003		1404	970	909	1008	1119	1002
HDL													
TG	24	25	38			25	20	26	29	23	34	20	27
CE	323	259	336			270	312	300	223	194	332	327	277

cholesteryl ester in HDL must be considered a coincidence, because there is no apparent reason for such a constancy.

Distribution of molecular species

The distribution of the carbon numbers of the cholesteryl esters, triacylglycerols and phosphatidylcholines in the various lipoprotein fractions from the individual normolipemic subjects are 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

TABLE V

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

Each value is an average of two to three estimates from replicate gas chromatographic analyses. Values given are percentages of lipid class.

Carbon numbers*	Males								Females				
	1	2	1690	1765	1767	1689	1858	Average	3	4	18	19	Average
VLDL													
36	35	33	33	37	36	36	30	34±2	34	36	40	35	36±2
38	36	40	41	40	39	41	45	40±3	40	37	40	38	39±2
40	29	27	26	23	25	23	25	25±2	26	27	20	27	25±3
43**	20	18	27	21	22	22	16	21±3	17	18	17	20	18±1
45**	80	82	73	79	78	78	84	79±8	83	82	83	80	82±1
46	ND***	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
48	4	5	8	6	7	8	8	7±2	6	9	5	5	6±2
50	19	19	18	19	19	19	19	19±0	25	24	21	17	22±3
52	55	51	40	52	50	48	47	49±5	48	47	48	52	49±2
54	20	22	30	20	22	23	22	23±3	18	17	24	23	21±3
56	2	3	4	3	2	2	4	3±1	2	3	2	3	3±1
LDL_2													
36	38	37	31	39	37	38		37±3	36	38	37	44	39±4
38	39	37	35	38	36	35		37±2	38	37	35	42	38±3
40	23	26	24	23	27	27		25±2	26	25	28	14	23±6
43	19	17	19	19	26	20		20±3	21	32	21	20	21±1
45	72	72	68	74	67	74		71±3	69	68	67	70	69±1
47	9	11	13	7	7	6		9±3	10	9	12	10	10±1
50	26	24	23	23	29	25		25±2	31	33	27	27	30±3
52	49	46	48	50	47	50		48±2	46	45	46	50	47±2
54	25	30	29	27	24	25		27±2	23	22	27	23	24±2
HDL													
36	37	36	32			35	32	34±2	34	38	36		36±2
38	40	41	41			39	40	40±1	41	39	40		40±1
40	23	23	27			26	28	25±2	25	23	24		24±1
43	19	18	16			22	16	18±3	21	24	19		21±3
45	69	72	71			68	72	70±2	66	61	71		66±5
47	12	10	13			10	12	11±1	13	15	10		13±3
50	29	14	28			29	23	25±6	20	25	30		25±5
52	53	61	49			53	45	52±6	60	55	57		57±3
54	18	25	33			18	32	25±7	20	20	13		18±4

*Carbon numbers 43—47 represent the cholesterol esters, with fatty acids of 16—20 acyl carbons; carbon numbers 36—40 represent diacylphosphatidylcholines with a total of 36—40 acyl carbons; carbon numbers 48—56 represent triacylglycerols with a total of 48—56 acyl carbons.

**Due to overlap with C_{44} triacylglycerol C_{47} cholesteryl ester has been omitted. However, analysis of fatty acid methyl esters (Table VI) indicates that C_{47} is <6%.

***ND, not determined.

three lipoprotein classes for any one subject. Likewise, closely similar carbon numbers are recorded for the triacylglycerols of all three lipoprotein classes for any one subject. The triacylglycerols of the VLDL were present in the highest amounts and provided the most detailed profiles of their structure. However, the LDL_2 triacylglycerols possessed a closely similar distribution for carbon numbers, when present in sufficient amounts for accurate analysis, as did the HDL fraction, which contained only minute quantities triacylglycerols. A simple determination of the carbon numbers, however, may not be adequate to demonstrate homogeneous populations of species of neither cholesteryl esters nor triacylglycerols for these lipoprotein fractions, because

TABLE VI

FATTY ACID COMPOSITION (MOLE %) OF CHOLESTERYL ESTERS OF VLDL, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF NORMOLIPEMIC SUBJECTS

Each value is an average of two replicate gas chromatographic analyses. Fatty acids identified by carbon number:double bond number.

Fatty acids	Males				
	1924	1690	1689	1858	Average
VLDL					
14:0	0.2		—	0.1	0.1±0.1
16:0	9.9		11.9	7.7	9.8±2.1
16:1	1.7		3.6	1.9	2.4±1.0
18:0	1.0		0.9	1.5	1.1±0.3
18:1	26.9		29.0	19.1	25.0±5.2
18:2	54.2		51.0	63.5	56.2±6.5
18:3/20:1	0.3		2.0	0.5	0.9±0.9
20:3	0.6		—	0.8	0.7±0.1
20:4	5.3		1.5	4.7	3.8±2.0
LDL_2					
14:0	0.6	0.3	0.4	0.4	0.4±0.1
16:0	14.3	11.0	11.8	10.7	12.0±1.6
16:1	2.2	2.3	2.3	1.6	2.1±0.3
18:0	1.0	0.9	0.6	0.9	0.9±0.2
18:1	21.7	20.5	20.1	14.3	19.2±3.3
18:2	53.2	59.2	60.2	67.2	60.0±5.7
18:3/20:1	0.5	0.5	0.4	0.2	0.4±0.1
20:3	0.6	0.8	0.3	0.4	0.5±0.2
20:4	5.9	4.2	3.8	4.3	4.6±0.9
HDL					
14:0	0.5	0.3	0.4	0.4	0.4±0.1
16:0	11.8	11.3	10.4	9.7	10.8±0.9
16:1	2.1	2.5	2.1	1.7	2.1±0.3
18:0	0.7	1.1	0.6	0.8	0.8±0.2
18:1	21.2	19.9	19.6	13.4	18.5±3.5
18:2	55.2	58.9	61.4	68.1	60.9±5.4
18:3/20:1	0.5	0.6	0.5	0.4	0.5±0.1
20:3	0.6	0.7	0.4	0.5	0.6±0.1
20:4	7.3	4.8	4.7	5.0	5.5±1.2

the saturated and the various unsaturated C_{18} fatty acid esters overlap and therefore may hide significant differences in the fatty acid composition of the esters. The true fatty acid compositions of the cholesteryl esters of VLDL, LDL_2 and HDL fractions from the same subjects are given in Table VI. It is seen that the cholesteryl esters of VLDL contain more oleic and less linoleic acid than do the cholesteryl esters of HDL, with those of LDL_2 possessing intermediate compositions. These results are in agreement with the data of Goodman and Shiratori [38] and of Morrisett et al. [39]. The latter observations are consistent with the relatively constant proportion of the C_{47} cholesteryl ester peak in the steryl ester fraction of all three lipoproteins. The fatty acid compositions of the triacylglycerols of the different lipoprotein classes of the same subjects are given in Table VII. It is seen that all lipoproteins contain comparable amounts of palmitic and linoleic acid in contrast

TABLE VII

FATTY ACID COMPOSITION (MOLE %) OF TRIACYLGLYCEROLS OF VLDL, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF NORMOLIPEMIC SUBJECTS

Each value is an average of two replicate gas chromatographic analyses. Fatty acids identified by carbon number:double bond number.

Fatty acids	Males				
	1924	1690	1689	1858	Average
VLDL					
14:0	1.9	1.6	2.0	2.8	2.1±0.5
16:0	28.2	26.7	31.3	26.2	28.1±2.3
16:1	4.0	4.4	4.7	4.6	4.4±0.3
18:0	5.1	5.5	2.8	2.0	3.8±1.7
18:1	42.7	41.0	41.4	32.6	39.4±4.6
18:2	16.2	18.1	16.1	29.7	20.0±6.5
18:3/20:1	1.1	1.8	1.2	1.4	1.3±0.3
20:3/20:4	0.9	0.9	0.5	0.8	0.8±0.2
LDL_2					
14:0	1.5	1.5	1.4	2.5	1.7±0.5
16:0	25.3	29.9	26.6	26.9	27.2±2.0
16:1	3.8	4.2	4.0	4.2	4.1±0.2
18:0	4.4	4.3	3.7	5.0	4.4±0.5
18:1	47.5	43.1	45.7	35.1	42.9±5.5
18:2	15.2	13.5	16.2	24.5	18.0±4.4
18:3/20:1	0.7	1.1	1.0	0.7	0.9±0.2
20:3/20:4	1.3	1.3	1.5	1.1	1.3±0.2
HDL					
14:0	1.7	1.7	1.7	2.8	2.0±0.5
16:0	24.9	28.0	26.6	25.6	26.3±1.3
16:1	4.2	4.8	4.3	4.6	4.5±0.3
18:0	4.4	4.3	2.9	2.5	3.5±1.0
18:1	46.4	45.4	45.1	36.8	43.4±4.4
18:2	16.0	13.9	17.2	25.4	18.1±0.1
18:3/20:1	0.8	0.9	1.1	0.8	0.9±0.1
20:3/20:4	1.6	1.2	1.1	1.6	1.4±0.3

to previous reports [38—40], where more palmitic and less linoleic acid were found in VLDL than in either LDL_2 or HDL.

Also the carbon number distributions for the diacylglycerol moieties of the phosphatidylcholines of the different lipoprotein classes when isolated from the same subjects were closely similar (Table V). The major carbon

TABLE VIII

CARBON NUMBER DISTRIBUTION (MOLE %) OF CERAMIDES OF VLDL, LDL_2 AND HDL PARTICLES OF FASTING PLASMA OF NORMOLIPEMIC SUBJECTS

Each value is an average of two replicate gas chromatographic analyses.

Carbon number*	Males				
	1924	1690	1689	1858	Average
VLDL					
32	1.6		3.1	2.2	2.3±0.8
33	1.1		1.9	1.2	1.4±0.4
34	37.9		39.1	35.2	37.4±2.0
35	1.6		1.6	1.7	1.6±0.1
36	9.4		8.3	9.4	9.0±0.6
37	0.6		0.7	1.1	0.8±0.3
38	3.7		3.7	4.2	3.9±0.3
39	0.9		1.6	1.8	1.4±0.5
40	9.4		11.1	13.0	11.2±1.8
41	4.5		5.5	5.3	5.1±0.5
42	29.4		23.5	25.0	26.0±3.1
LDL_2					
32	2.5	3.1	4.3	2.7	3.2±0.8
33	1.6	1.8	2.3	1.6	1.8±0.3
34	35.1	31.5	32.2	34.1	33.5±1.5
35	2.0	1.3	1.5	1.2	1.5±0.4
36	8.6	7.5	7.3	6.7	7.5±0.8
37	0.6	0.5	0.9	0.6	0.7±0.2
38	4.6	6.0	5.4	5.0	5.3±0.6
39	1.2	2.0	2.1	1.8	1.8±0.4
40	12.4	16.1	14.7	15.9	14.8±1.7
41	4.8	5.6	6.2	5.5	5.5±0.6
42	26.6	24.5	22.0	25.0	24.5±1.9
HDL					
32	1.7	2.7	3.5	1.8	2.4±0.8
33	0.9	1.4	1.6	1.0	1.2±0.3
34	24.3	24.5	26.1	23.0	24.4±1.3
35	0.6	0.9	1.1	0.8	0.9±0.2
36	6.9	6.5	6.8	5.8	6.5±0.5
37	0.1	0.5	0.5	0.4	0.4±0.2
38	4.2	6.4	5.4	5.6	5.4±0.9
39	0.9	1.9	2.1	1.8	1.7±0.5
40	15.3	19.0	17.7	20.5	18.1±2.2
41	6.3	5.5	6.2	6.0	6.0±0.4
42	38.7	30.5	29.0	33.2	32.9±4.3

*Total number of carbons in the fatty acid and the nitrogenous base of the ceramide.

numbers were 36—40 in about the same proportions in all fractions. We have shown elsewhere [41] that the individual molecular species of the phosphatidylcholines are very nearly identical in the different lipoproteins. It was noted, however, that the arachidonoyl species were present in a slightly higher proportion in the HDL_3 fractions, when compared to the corresponding LDL and VLDL fractions from the same subjects.

In agreement with previous findings [24] marked differences, however, were seen in the composition of the molecular species of the sphingomyelins of the different lipoprotein classes when isolated from the same subjects. Table VIII shows the differences in the carbon number composition of the ceramides of the VLDL, LDL_2 and HDL fractions from several subjects. It is seen that the HDL fraction contains a significantly higher proportion of the longer chain species ($C_{24:0}$ and $C_{24:1}$ amides of sphingosine) and a lower proportion of the shorter chain species ($C_{16:0}$ amides of sphingosine) than the VLDL and LDL_2 fractions, which differ little from each other. Detailed analyses of the molecular species have revealed minor differences in the proportions of the nitrogenous bases [24]. For the present purposes it is sufficient to note that the HDL fraction is either not directly derived from the VLDL or it has been extensively diluted by HDL from a different origin.

DISCUSSION

Validity of analytical measurements

The accuracy and precision of the GLC measurements of cholesterol and triacylglycerol [21] as well as of phospholipids [25] have been documented in previous publications and are further indicated in the present work by the overall consistency of the data and a good agreement between the GLC results and the results of conventional measurements reported in the literature [28]. It may therefore be concluded that the lipid class compositions of the various lipoproteins are essentially correct. Also the detailed accounts of the molecular species of the phosphatidylcholines and sphingomyelins necessary for evaluating the precursor—product relationships among the plasma lipoproteins must be essentially correct. The general results obtained in the present comparisons correspond closely to those made earlier on similar preparations of lipoproteins, which had led to comparable conclusions [31,33,40]. Likewise, the differences demonstrated in the C_{18} fatty acid compositions in a limited number of subjects among the plasma lipoprotein cholesteryl esters must be correct, as they correspond to similar observations made in those earlier studies where comparisons were made among samples obtained from the same individuals [39]. The identity implied among the triacylglycerol populations of the lipoprotein classes on the basis of the carbon number distribution and fatty acid composition, however, must be further confirmed by stereospecific analyses of the triacylglycerol structure. We are currently in the process of completing such comparisons and the results will be published elsewhere [42]. The data obtained to date suggest that the triacylglycerols of the VLDL and LDL_2 possess closely similar diacylglycerol moieties and therefore similar enantiomeric structures. The enantiomeric nature of the triacylglycerols of HDL has not been assessed. Since marked differences have been noted

between the enantiomeric structures of the triacylglycerols of chylomicrons and VLDL [42] there remains a need for a more detailed assessment of the equilibration of the triacylglycerols among the plasma lipoprotein classes. Nevertheless, the detailed knowledge of the steryl ester and the choline-containing phospholipid composition representing the neutral lipid core and the surface material of the lipid particle, respectively, is sufficient for at least a partial assessment of the hypothesis of Eisenberg et al. [9].

Furthermore, the lipid class composition provides characteristic lipid class ratios from which the particle sizes can be calculated assuming a lipid core model for all lipoproteins [43]. The calculated values are in the range of the particle sizes derived for these lipoproteins from direct measurements [29,30].

Validity of hypothesis

If a direct precursor—product relationship exists among the plasma VLDL, LDL and HDL lipoproteins, as postulated by Eisenberg et al. [9], it would be anticipated that certain similarities would be found in the mass composition of the lipids between the corresponding lipoprotein fractions. This should be especially so if these cascade-like transformations of lipoproteins took place largely in the plasma compartment with only the concentration of the starting materials and the end-products being modified by the tissues, as claimed on the basis of in vitro studies [11,44]. The interconversion hypothesis predicts that during lipoprotein lipase mediated triacylglycerol hydrolysis, VLDL particles become progressively smaller, change their protein and lipid composition and through a stage of formation of intermediate particles (IDL) (d = 1.006) are converted to plasma LDL. Kinetic studies carried out in normal human subjects have substantiated the scheme and have demonstrated that within experimental error all of the major structural apoprotein of VLDL (apo B) is converted to LDL, and that all of the LDL-apo B originated from VLDL [1,2]. During the process both core and surface components of the VLDL are being affected, resulting in a differential recovery in the LDL end-products. In vitro studies have suggested [11,45] that the cholesteryl ester remains with the residual VLDL triacylglycerols (and apo B) even when 70—80% of the triacylglycerols are hydrolyzed; thereafter they are quantitatively recovered within the particles of the LDL range. In the absence of any known mechanism of synthesis of cholesteryl esters in the VLDL particles, it would be anticipated that both VLDL and LDL_2 would have identical cholesteryl ester compositions. An examination of the carbon number distribution revealed only minor differences, while an assessment of the component fatty acids indicated a significantly higher unsaturation of the LDL_2 cholesteryl esters. In fact, the cholesteryl esters of the LDL_2 fraction resembled more those of the HDL than those of the VLDL. Such a differential distribution of the cholesteryl esters suggests that in addition to a direct transfer of the cholesteryl esters from VLDL to LDL_2 other processes must take place to account for the observed mass distribution. Chajek and Fielding [46] have recently isolated from human plasma a cholesteryl ester transfer protein, which stoichiometrically exchanges one molecule of HDL cholesteryl ester for one molecule of LDL triacylglycerol. Although the significance of this transfer is not known, it could possibly account for an increased unsaturation of LDL_2 cholesteryl esters and the presence

of triacylglycerols in HDL, especially if the HDL cholesteryl esters, which are derived via the lecithin:cholesterol acyltransferase [7] were more unsaturated than those of VLDL or LDL_2. It is also possible that at least a partial equilibration of the cholesteryl esters of LDL_2 and HDL could have taken place by fusion of some HDL particles with either VLDL or LDL_2, as postulated by Anderson et al. [47], Grow and Fried [48] and Tall and Small [10]. On the basis of the similarity in the fatty acid composition it is unlikely that the triacylglycerols of VLDL, LDL_2 and HDL would differ significantly in the composition of their molecular species. An absence of triacylglycerol differences would be consistent with a limited preferential hydrolysis of the unsaturated triacylglycerols by lipoprotein lipase [49]. The residual triacylglycerols of VLDL found in LDL would be therefore anticipated to be similar to those of the original VLDL. Furthermore, the triacylglycerols of HDL could have been derived from LDL via exchange for cholesteryl esters, as postulated by Chajek and Fielding [46], without any change in the composition of their molecular species. No change in molecualr species of the triacylglycerols would also be expected if any triacylglycerols of HDL were eventually returned to LDL_2 and/or VLDL by fusion with any unstabilized HDL particles, as postulated by Tall and Small [10]. Finally, the recently characterized triacylglycerol exchange protein of Barter et al. [50] could also have contributed to the equilibration of the triacylglycerols among all three lipoproteins. Nevertheless, on account of the differences in the cholesteryl ester composition and in cholesteryl ester/triacylglycerol ratios, even the composition of the neutral lipid cores of the VLDL, LDL_2 and HDL particles cannot be fully accounted for by a simple precursor—product relationship, as postulated by Eisenberg et al. [9] and Tall and Small [10], and that plausible additional mechanisms must be invoked to establish the experimentally observed mass distributions.

According to the hypothesis, the LDL particles formed from VLDL retain only sufficient surface material to provide a monolayer coating the entire surface of the sphere, the rest of the surface material being partly hydrolyzed by phospholipase A_2 [51] and partly shed possibly in the form of LPX-like particles [52]. In this process the apo B peptide is retained exclusively on the LDL particles, while the LPX-like aggregates acquire apo A_1 from preformed HDL. The present results indicate that the surface material retained on the LDL is either subject to a preferential loss of phosphatidylcholine due to hydrolysis by phospholipases or there has been a preferential retention of sphingomyelin on the LDL surface during the shedding of the excess surface material in the form of LPX. It is possible that a partial phase separation has taken place during the formation of the LPX-like aggregates, which could have excluded much of the sphingomyelin from the more organized liposomal structure. Phase separation of phosphatidylcholine and sphingomyelin is known to take place under certain experimental conditions [53], but it is not known to what extent, if any, it may take place under physiological conditions. On the basis of physicochemical experiments, Calhoun and Shipley [54] have suggested that a likely candidate for inducing lateral phase separations is a long chain fatty acid ($C_{24:0}$ or $C_{24:1}$) ceramide linked to phosphorylcholine. Should such a phase separation take place during the LPX formation, it could provide a plausible explanation for an eventual enrichment of sphingomyelin in the

LDL_2 and of the longer chain sphingomyelins in the HDL end-product of the excess VLDL surface material. Alternative explanations for the relative enrichment of sphingomyelin in the LDL fraction may be advanced on the basis of preferential binding of this phospholipid by apo B, or a preferential binding of phosphatidylcholine by the apo A_1 transferred to the intermediate LPX from HDL. The available information would suggest that apoprotein A_1 must interact with apoprotein A_2 for a significant phospholipid binding [55,56], but it is not known whether or not any distinction is made between the two types of choline phospholipids. There is not sufficient long chain sphingomyelin in either the liver or intestinal sources of nascent HDL to account for the observed distortion of the molecular species of the ceramides, although the red blood cells could have contributed some of it [57]. In any event, a direct precursor—product relationship, as previously put forward [9,10], cannot be immediately reconciled with the differences in the composition of the molecular species of the sphingomyelins in the VLDL and HDL fractions, and with the differences in the sphingomyelin/phosphatidylcholine ratios in the VLDL, LDL_2 and HDL fractions. Additional mechanisms or alternative series of transformations must be postulated in order to account for the characteristic lipid mass distribution.

The formation of HDL from VLDL is believed to be completed via the action of lecithin:cholesterol acyltransferase, which catalyzes the esterification of free cholesterol by phosphatidylcholine in the LPX monolayer in the presence of apo A_1 resulting in a transfer of the cholesteryl ester into the interior of the phospholipid bilayer [9,10]. According to Tall and Small [10] either the LPX or the discoidal lamellar aggregates derived from it via apo A_1 serve as substrates for lecithin:cholesterol acyltransferase. The lysophosphatidylcholine is relased into the medium where it is presumably bound by albumin. With a gradual depletion of the free cholesterol and phosphatidylcholine the surface area of the aggregate gradually shrinks and the interior is filled with cholesteryl esters resulting in a spherical particle of a diameter characteristic of the apo A_1 containing lipoproteins [34]. According to this scheme the fatty acid composition of the HDL cholesteryl esters should resemble that of the sn-2-position of the HDL phosphatidylcholines. In fact, the cholesteryl esters of the HDL are somewhat more saturated than would have been anticipated from the fatty acid composition of the component phosphatidylcholine. Perhaps during the transfer of the cholesteryl esters from HDL to LDL via the cholesteryl ester transfer protein the more unsaturated species are preferentially removed [46]. It was already noted, however, that the reciprocal transfer of triacylglycerols from LDL to HDL could not have involved a preferential transfer of the more unsaturated triacylglycerols. According to the hypothesis, the HDL should have been essentially free of triacylglycerols. The analytical results, however, demonstrate that triacylglycerols can be reproducibly recovered as a consistent and persistent component of the ultracentrifugally isolated HDL fraction.

CONCLUSIONS

The present evaluation of the plasma lipoprotein mass composition of the lipid classes and molecular species from the same individuals reveals a great

similarity, which may have been achieved either by a non-specific equilibration of the components or by an interconversion of the lipid particles, or both. A direct conversion of VLDL into LDL, via lipoprotein lipase, and of the excess surface material of the VLDL into HDL, via lecithin:cholesterol acyltransferase, however, would appear unlikely in view of the discrepancies in the lipid class and molecular species composition of the phospholipids. If the hypothesis of the lipoprotein interconversion is correct, the various plasma lipoproteins must have undergone such other transformations as those brought about by lysophosphatidylcholine transacylase, phospholipase A_2, cholesteryl ester-triacylglycerol, triacylglycerol, and phospholipid exchange protein, as well as by such physicochemical phenomena as lateral phase separation and preferential binding of phospholipids by specific apoproteins. It is possible that these phenomena have been especially active in the equilibration of the lipids of the major fasting plasma lipoproteins. It would therefore be of interest to perform a comparable examination of the lipid class and molecular species composition on the plasma lipoproteins in the postabsorptive state of normal subjects, as well as of fasting and postabsorptive patients with hyperlipoproteinemia. Ultimately the validity of the lipoprotein interconversion hypothesis should be examined by means of specifically labelled lipid molecules over appropriate periods of time in order to establish any precursor—product relationships of individual lipoprotein classes, as well as the time course of non-specific equilibration of lipid classes and molecular species. Hopefully, the sensitive methods of detailed lipid analysis described here along with measurements of stable or radio-isotope distribution will be adequate for this task.

ACKNOWLEDGEMENTS

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

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