Composition, concentration, and size of low density lipoproteins and of subfractions of very low density lipoproteins from serum of normal men and women

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Abstract Low density lipoprotein (LDL) and four subfractions, A, B, C, and D of very low density lipoprotein (VLDL), characterized by the following decreasing S_f values, >400, 175–400, 100–175, and 20–100, respectively, were isolated by density gradient preparative ultracentrifugation from serum of normal men and women and analyzed for lipids, total protein, and apoB. The percentage distribution of the triglycerides of VLDL on fractions A to D were 1, 25, 30, and 45%, respectively, for both males and females. The numbers of VLDL particles for males in fractions B to D were 5, 10, and 40 × 10^{12} per ml serum, respectively. Females, who had lower VLDL concentrations than males, had half as many particles per ml of serum. The number of LDL particles was similar in males and females and was 10 times that of VLDL. The relative composition of the lipoproteins changed progressively from fraction B to D and from D to LDL. The percentage of triglyceride and soluble proteins fell, due to loss of mass of these constituents from the particles. The percentage of apoB and cholesteryl esters rose; this was not due to an increase of mass but because the particles became smaller. There were no sex differences. The numbers of molecules per particle of the constituents showed a successive decrease from VLDL-B to LDL for triglycerides, free cholesterol, phospholipids, and soluble apolipoproteins, while it remained constant for apoB, as well as for cholesteryl esters from VLDL-C to LDL. These data fit the following hypothesis for the VLDL to LDL cascade. The large VLDL particle successively loses molecules of triglycerides (core) and of soluble protein, free cholesterol, and phospholipids (surface). In this process, VLDL retains its molecules of apoB, and cholesteryl esters are lost from large VLDL but not from medium or small-sized VLDL.—**Kuchinskiene, Z., and L. A. Carlson**. Composition, concentration, and size of low density lipoproteins and of subfractions of very low density lipoproteins from serum of normal men and women. *J. Lipid Res.* 1982. **23:** 762–769.

Supplementary key words cholesterol • triglycerides • phospholipids • apolipoprotein B • soluble apolipoproteins • molecular composition

Many studies have reported increased concentrations of low density (LDL) and/or of very low density (VLDL) lipoproteins in blood serum of patients with ischemic vascular diseases. In these studies the concentration of one or, at most, two components of the lipoproteins, total cholesterol and triglycerides, have been analyzed. However, the lipoprotein particles have a much more complex composition and consist of a *core* containing cholesteryl esters and triglycerides and a *surface shell* in which there are phospholipids, free cholesterol, and proteins (apolipoproteins) (1–3). A complete characterization of lipoprotein abnormalities in various diseases hence requires analysis of all the components of the VLDL and LDL particles.

Furthermore, available knowledge of lipoprotein metabolism has led to the theory that LDL is formed from VLDL, that larger VLDL particles become smaller VLDL, that *one* VLDL particle eventually is transformed into *one* LDL particle, and that this process occurs intravascularly (3). Therefore it is of interest to know the complete composition of larger and smaller VLDL and of LDL in man in various conditions. Human VLDL has been subfractionated by various methods, viz. ultracentrifugation at progressively increasing centrifugal forces with repeated washings (1), ultracentrifugation through a density gradient (4, 5), zonal ultracentrifugation (6), and by a combination of preparative ultracentrifugation and gel chromatography (2). The isolated VLDL fractions have been analyzed with regard to various constituents and physicochemical characteristics. Only one of these studies has included a complete chemical analysis, including apolipoprotein B (apoB) (5), and none had sufficient control material either to establish normal values for VLDL subfractions or to permit evaluation of sex differences.

In the present study we report data on the content of

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apoB, apolipoprotein B.

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triglycerides, cholesteryl ester, free cholesterol, phospholipids, total protein, apoB, and isopropanol-soluble proteins in four VLDL subfractions, characterized by decreasing size, and in LDL from normal men and women. Using one fixed model for the lipoprotein structure, the data also permitted calculations of lipoprotein size, number of lipoprotein particles, molecular weight, and molecular mass of lipoprotein constituents.

MATERIALS AND METHODS

Blood was taken in the morning, after an overnight fast, from apparently healthy men (n = 14, mean age 53 years, range 34–80), and women n = 18, mean age 52 years, range 24–71) who participated in other ongoing studies. The majority were randomly selected from Stockholm. All were in good health, living their ordinary lives, and none was on chronic medication or diet. Ordinary routine laboratory tests were normal and no endocrine disorders were present.

Blood was allowed to clot at room temperature for 2 hr, serum was recovered by low speed centrifugation (3600 rpm), and EDTA was added for the routine analysis of serum lipoproteins as described in detail elsewhere (7). In principle VLDL was floated to the top by ultracentrifugation at d 1.006 kg/l, whereafter LDL and other apoB-containing lipoproteins were precipitated by heparin/manganese, leaving high density lipoproteins (HDL) in solution. The fractions were extracted with chloroform–methanol and analyzed for total cholesterol and triglycerides.

Methods

VLDL was subfractionated by cumulative rate centrifugation as described by Lindgren, Jensen, and Hatch (4). We have reported the procedure in detail (5). In short, serum was added with solid NaCl to increase the density to 1.10 kg/l and a 4-ml sample was transferred to a cellulose nitrate centrifuge tube. Three ml of d 1.065, 3 ml of d 1.020, and 3.4 ml of d 1.006 kg/l NaCl solutions were layered above. Ultracentrifugation was carried out in a SW 40 Ti rotor at 20°C. Four consecutive runs were done, calculated to float VLDL particles of the following diameters to the top of the tube: fraction A, >75 nm (S_f > 400); fraction B, 50–75 nm (S_f 175–400); fraction C, 37–50 nm (S_f 100–175); and fraction D, 20–37 nm (S_f 20–100). Each fraction was recovered by careful aspiration of the top 1 ml after each run and the tube was then refilled with 1 ml of d 1.006 kg/l solution. After the fourth and final centrifugation, the LDL was easily seen as a yellow band about 5–8 mm wide located 45 to 55 mm from the bottom. This isolated LDL was also recovered by aspiration. Twelve ml of serum, i.e., in three centrifuge tubes, were needed to get sufficient material for all analyses, and the fractions from the three tubes were pooled.

Analyses

Before analysis the lipoprotein fractions were transferred to volumetric flasks. Lipids were extracted with chloroform–methanol (8). Aliquots of the chloroform phase were used for analysis of triglycerides (in duplicate) (9) and phospholipids (in triplicate) as phosphorus (10) after wet combustion; triolein (Sigma, St. Louis, MO, USA) and KH_2PO_4 were the respective standards. Free and esterified cholesterol were determined directly on 0.001 to 0.1 ml of the lipoprotein fractions, in triplicate, with an enzymatic method (Mercotest, E Mercl, Darmstadt, Germany). Dilutions of pooled human serum were used as standards, and standard curves of the second degree (curvilinear) were fitted to the standards by the method of least squares. The content of free and esterified cholesterol in the standard was determined by the Sperry-Webb technique (11). Total protein was estimated in triplicate on aliquots of the lipoprotein fractions by the method of Lowry et al. (12). All samples, including the standards, were extracted with chloroform after color development to remove any turbidity. Soluble proteins were estimated after extraction (and concomitant precipitation of apoB) with isopropanol (13, 14) for VLDL and isobutanol for LDL[3] by the same method. The content of apoB was calculated as the difference between total and soluble protein (14). Bovine serum albumin was used as protein standard. Lipoprotein electrophoresis was performed in agarose gel according to Noble (15).

Recovery

Runs where the recovery of VLDL triglycerides (sum of VLDL subfractions) was less than 70% were not accepted. The mean recoveries were (males/females): VLDL triglycerides, 87/91%; VLDL cholesterol, 91/89%; LDL triglycerides, 54/58%; and LDL cholesterol, 89/90%.

Calculations

Dimensions of lipoprotein particles such as diameters, molecular weight, S_f-value, and particle numbers were calculated as described in detail elsewhere (5). In essence these calculations were based on the assumption that lipoproteins are spherical particles with triglycerides and cholesteryl esters in the core and a constant thickness of the polar surface shell of 2.15 nm (2). From calculations of total lipoprotein volume and core volume, using the partial specific densities, the diameter is easily obtained

[3] Holmquist, L. Personal communication.

TABLE 1. Cholesterol and triglyceride concentration in serum, VLDL, LDL, and HDL for males and females

	Males	Females
	mmol/l ± SEM	
Total cholesterol		
Serum	6.42 ± 0.37	6.89 ± 0.27
VLDL	0.46 ± 0.05*	0.31 ± 0.03
LDL	4.38 ± 0.28	4.36 ± 0.20
HDL	1.38 ± 0.07**	1.83 ± 0.09
Triglycerides		
Serum	1.33 ± 0.12	1.13 ± 0.08
VLDL	0.77 ± 0.10	0.55 ± 0.06
LDL	0.36 ± 0.03	0.38 ± 0.03
HDL	0.15 ± 0.02	0.15 ± 0.008

Differences between the sexes were tested by Student's *t* test; *P* value for difference: *, <0.05; **, <0.001.

(2, 5). The molecular weight and other characteristics were then calculated as described (5). The molecular mass of the lipoprotein constituents in daltons was calculated as weight fraction times molecular weight for the different particle fractions. The number of molecules per particle was then obtained by division by the molecular weight. Since the apolipoproteins in the lipoproteins have widely different molecular weights, the protein content was expressed as the number of amino acids per particle, using an average residue weight of 100. Statistical calculations were as recommended by Snedecor (16).

All calculations were performed on a minicomputer (Alfa LSI, Computer Automation, CA, USA) equipped with a computer display terminal (Tektronix, OR, USA). The data were stored on magnetic disks. Programs were written in Basic specifically for the treatment of these data.

RESULTS

The lipoprotein concentrations for the 14 men and the 18 women are given in **Table 1**. Serum total cholesterol as well as LDL cholesterol were similar in the two sexes. As expected, the women had significantly higher HDL cholesterol than the men. Also, as expected, males had higher VLDL levels than females.

A typical electrophoretic pattern of the isolated lipoproteins is shown in **Fig. 1**. There was no material visible in the VLDL-A fraction defined to contain only chylomicrons. The VLDL-B and VLDL-C fractions both had "rapid" pre-β mobility, while the D fraction had a slower pre-β mobility. The LDL fraction showed a discrete band with β-mobility. The sinking pre-β (or Lp(a)) lipoprotein, present in both whole serum and in the bottom fraction after ultracentrifugation at d 1.006 kg/l, was not present in the LDL fraction isolated by density gradient centrifugation.

Concentrations of lipoprotein constituents

The concentrations of the constituents of the lipoproteins are given in **Table 2**. The concentration of all components increased for each VLDL fraction going from larger to smaller particles in both sexes. The increase was, however, quite different for various constituents. The smallest rise was for triglycerides, less than twofold from VLDL-B to VLDL-D, while the largest increase was for apoB, which increased tenfold. These differences are explained by the striking disparities in composition between the VLDL fractions as discussed below.

VLDL-C and VLDL-D fractions from males had higher concentrations of all constituents than those found in the fractions from females. The men also had higher values for VLDL-B than the women but this was not statistically significant.

In several normolipidemic subjects, as might be expected, there was no detectable material in VLDL-A. Its triglyceride contributed only 1–2% to the total VLDL triglyceride. The VLDL triglyceride was distributed in VLDL-B, VLDL-C, and VLDL-D with about 25, 30, and 45%, respectively, for both men and women. For VLDL cholesterol the corresponding figures were 10, 15, and 75%, respectively. The ratio cholesterol/triglycerides, often used in the characterization of VLDL, increased progressively from VLDL-B to VLDL-D (Table 2).

The isolated LDL had concentrations of its components strikingly different from the VLDL fractions. In particular the concentrations of apoB and of total cholesterol were about tenfold that in VLDL-D. There were

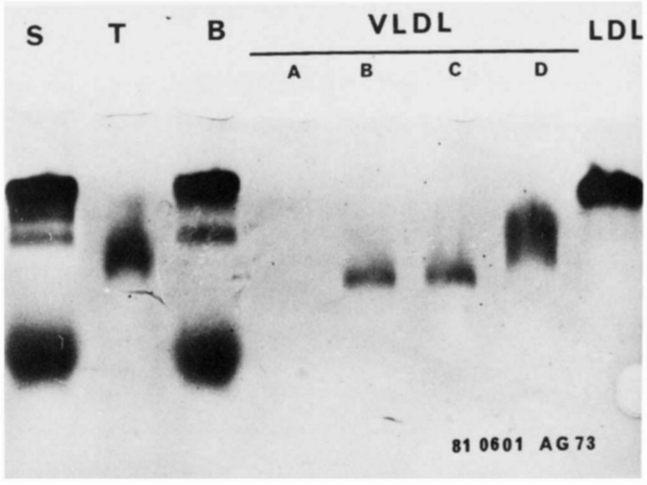

Fig. 1. Agarose gel lipoprotein electrophoresis of the isolated VLDL and LDL fractions, whole serum (S), and the top (T) and bottom (B) fractions after ultracentrifugation at d 1.006 kg/l in the 40.3 rotor (the same serum for all samples). A rather heavy, sinking pre-β lipoprotein is visible in the bottom (B) fraction together with normal appearing β- and α-lipoprotein bands. There is no visible sinking pre-β band in the isolated LDL fraction.

TABLE 2. Concentration of constituents in the four VLDL fractions and the LDL fraction in males and females

Constituents	Sex	VLDL A	VLDL B	VLDL C	VLDL D	LDL
Total protein	M	1.45 ± 1.10[a]	24.4 ± 5.00	40.0 ± 5.75**	107.3 ± 8.50***	997 ± 73.8
mg/l	F	0.07 ± 0.07	13.8 ± 1.84	22.1 ± 2.13	70.6 ± 3.37	999 ± 66.5
Soluble protein	M	0.89 ± 0.63	18.0 ± 3.32	26.1 ± 3.19**	42.4 ± 3.35***	11.03 ± 1.30
mg/l	F	0.06 ± 0.06	10.68 ± 1.41	15.4 ± 1.43	26.4 ± 2.08	9.17 ± 0.72
ApoB	M	0.58 ± 0.48	6.50 ± 1.74	13.8 ± 2.62*	64.9 ± 5.46**	986 ± 73.6
mg/l	F	0.009 ± 0.009	3.04 ± 0.59	6.62 ± 0.83	44.2 ± 2.38	990 ± 66.2
Total cholesterol	M	0.003 ± 0.001	0.04 ± 0.01	0.08 ± 0.01**	0.31 ± 0.02***	3.69 ± 0.20
mmol/l	F	0.003 ± 0.001	0.02 ± 0.003	0.03 ± 0.004	0.20 ± 0.02	3.82 ± 0.17
Free cholesterol	M	0	0.016 ± 0.006	0.03 ± 0.006***	0.11 ± 0.010***	0.94 ± 0.06
mmol/l	F	0	0.007 ± 0.002	0.01 ± 0.002	0.07 ± 0.006	0.98 ± 0.06
Cholesteryl esters	M	0.003 ± 0.001	0.03 ± 0.008**	0.04 ± 0.009*	0.20 ± 0.02**	2.74 ± 0.16
mmol/l	F	0.003 ± 0.001	0.01 ± 0.002	0.02 ± 0.002	0.13 ± 0.009	2.84 ± 0.12
Phospholipids	M	0.001 ± 0	0.06 ± 0.01	0.08 ± 0.01**	0.19 ± 0.02**	0.95 ± 0.06
mmol/l	F	0	0.03 ± 0.004	0.04 ± 0.004	0.12 ± 0.007	0.98 ± 0.03
Triglycerides	M	0.008 ± 0.002	0.19 ± 0.03	0.23 ± 0.03*	0.37 ± 0.03***	0.22 ± 0.02
mmol/l	F	0.010 ± 0.003	0.12 ± 0.02	0.13 ± 0.02	0.23 ± 0.01	0.22 ± 0.01
Ratio cholesterol	M		0.23 ± 0.02	0.34 ± 0.02*	0.88 ± 0.05	18.1 ± 1.66
to triglycerides	F		0.20 ± 0.02	0.27 ± 0.01	0.91 ± 0.04	18.3 ± 1.46

[a] Mean ± SEM.
Differences between sexes were tested by Student's *t* test; *P* value for the difference: *, <0.05; **, <0.01; ***, <0.001.

no significant sex differences in concentrations with regard to any of the components of LDL.

Relative composition of lipoproteins

The relative compositions of the different lipoprotein fractions are given in **Table 3** and summarized for the females in **Fig. 2**. From the largest VLDL fraction to LDL there were progressive changes. The most pronounced changes were the decrease in triglycerides from about 66 to 5%, the increase in cholesteryl esters from 7.6 to 44% and the rise for apoB from about 2 to 24%. The amount of soluble proteins fell from 7 to 0.2%. However, as will be described below, while the fall in the percentage of triglycerides and soluble proteins was due to actual loss of mass of these constituents from the lipoprotein particle, the rise in the percentage of cholesteryl esters and apoB was due not to an increase of their mass in the lipoprotein particle but rather to the fact that

TABLE 3. Percentage composition of VLDL-B, VLDL-C, VLDL-D, and LDL[a]

Constituents	Sex	VLDL B	VLDL C	VLDL D	LDL
ApoB	M	2.33 ± 0.27	3.81 ± 0.23	8.82 ± 0.25	24.1 ± 0.75
	F	2.27 ± 0.45	3.63 ± 0.26	9.47 ± 0.35	23.5 ± 0.81
Soluble protein	M	7.63 ± 0.38	8.04 ± 0.32	5.78 ± 0.15	0.3 ± 0.03
	F	7.57 ± 0.33	8.53 ± 0.30	5.58 ± 0.29	0.2 ± 0.02
Free cholesterol	M	1.97 ± 0.28	3.15 ± 0.31	5.82 ± 0.28	9.05 ± 0.26
	F	1.35 ± 0.27	2.30 ± 0.27	5.78 ± 0.27	9.02 ± 0.27
Cholesteryl esters	M	7.72 ± 1.1	9.50 ± 0.83	18.1 ± 0.98	44.0 ± 0.71
	F	7.58 ± 1.13	8.44 ± 0.72	18.3 ± 0.68	44.7 ± 0.80
Phospholipids	M	15.4 ± 0.45	17.0 ± 0.49	19.2 ± 0.41	17.8 ± 0.75
	F	14.2 ± 0.46	16.5 ± 0.34	19.5 ± 0.47	17.9 ± 0.50
Triglycerides	M	65.0 ± 1.3	58.5 ± 0.92	42.3 ± 0.71	4.77 ± 0.39
	F	67.1 ± 1.06	60.6 ± 0.75	41.4 ± 0.81	4.76 ± 0.33

[a] Mean percentage ± SEM.

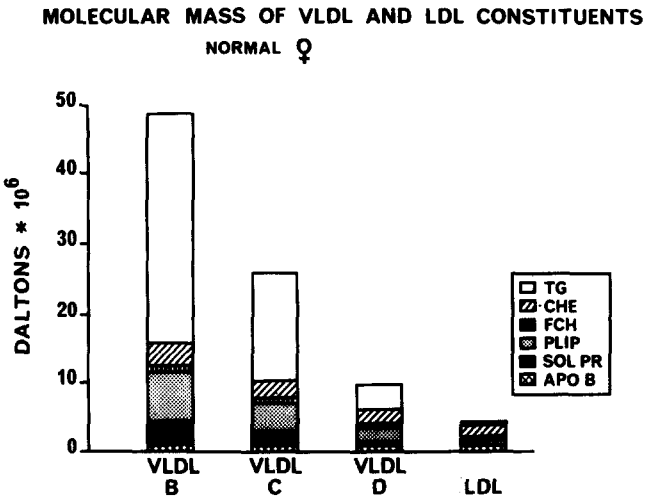

Fig. 2. Percentage composition of female VLDL-B, VLDL-C, VLDL-D, and LDL. TG, triglycerides; CHE, cholesteryl ester; FCH, free cholesterol; PLIP, phospholipid; SOL PR, soluble apolipoproteins.

the particle became smaller as a result of the loss of the other constituents. There were no sex differences for the relative composition.

Particle dimensions

Table 4 shows various calculated particle dimensions. The diameters, the basically calculated parameter, for the different VLDL fractions agreed well with the predicted values except for male VLDL-B. Here two men had diameters of about 40 nm instead of >50 nm. They each had a lower value of nonpolar lipid, about 70% as compared to 77% for the other men. This may be an analytical error due to the low concentrations in this fraction or it may be real. These two men had, however, normal values for both VLDL-C and VLDL-D. The presence of these two low values explains the significant sex difference in Table 4 concerning the lower relative amount of apolar lipids for males only in VLDL-B.

The VLDL-B and VLDL-C were smaller for men than for women. Therefore S_f values and molecular weights of the particles in these fractions were also smaller for the males.

The number of particles doubled from VLDL-B to VLDL-C, and the increase in going to VLDL-D was about fivefold. LDL had about 10 times as many particles as VLDL-D. Men had about twice as many VLDL particles as women. There was no difference in the number of LDL particles.

Molecular composition

The number of molecules of each constituent per lipoprotein particle is given in **Table 5,** and **Fig. 3** summarizes the data for females. Going from VLDL-B to VLDL-C to VLDL-D and eventually to LDL, the molecular weight decreased successively from 50 to 4 million daltons. The difference in number of molecules comparing one lipoprotein class to the next larger class showed a consistent pattern from class to class with a decrease in triglycerides, free cholesterol, phospholipids, and soluble protein. The most dramatic decreases, comparing LDL to VLDL-D, were for triglycerides and soluble protein, being 96 and 98% lower, respectively. ApoB had the same number of amino acid residues in all four fractions and the number of molecules of cholesteryl ester was the same in VLDL-C, VLDL-D and LDL, while it was higher in VLDL-B.

There were no sex differences with regard to the number of molecules of the different constituents for LDL and the smallest VLDL. For VLDL-B and VLDL-C, however, males had smaller numbers of triglycerides, phospholipids, and soluble protein. This is readily explained by the fact that these two lipoprotein fractions were smaller for men than for women.

DISCUSSION

The theoretical aspects of the procedure used to subfractionate VLDL, i.e., cumulative rate centrifuga-

TABLE 4. Some calculated physical and physicochemical dimensions of VLDL-B, VLDL-C, VLDL-D, and LDL[a]

Dimensions	Sex	VLDL B	VLDL C	VLDL D	LDL
Diameter, nm	M	49.4 ± 1.49*	41.0 ± 0.67*	31.9 ± 0.48	22.7 ± 0.37
	F	54.2 ± 0.96	43.5 ± 0.91	31.2 ± 0.48	23.1 ± 0.44
S_f value	M	133 ± 9.31*	80.9 ± 3.21*	38.6 ± 1.43	6.42 ± 0.61
	F	163 ± 6.71	93.3 ± 4.88	36.4 ± 1.52	7.48 ± 0.92
Molecular weight × 10^5	M	378 ± 30.6**	214 ± 10.7*	102 ± 4.85	39.0 ± 1.91
	F	487 ± 23.7	256 ± 14.8	96.7 ± 4.29	41.3 ± 2.39
Number of particles per ml × 10^{12}	M	4.9 ± 1.59	9.9 ± 1.9**	40.9 ± 4.3**	584 ± 44
	F	2.13 ± 0.34	4.29 ± 0.4	27.2 ± 1.61	582 ± 42.6

[a] Mean value ± SEM.
Sex differences were tested and, when present, are indicated as in Table 2.

TABLE 5. Number of molecules × 10^2 of constituents per lipoprotein particle

Constituents	Sex	VLDL B	VLDL C	VLDL D	LDL
Triglycerides	M	294 ± 28*	146 ± 9*	50 ± 2	2.2 ± 0.2
	F	386 ± 19	184 ± 12	47 ± 3	2.3 ± 0.2
Cholesteryl esters	M	35 ± 4	30 ± 2	29 ± 3	27 ± 2
	F	50 ± 8	32 ± 4	27 ± 2	29 ± 2
Free cholesterol	M	21 ± 2	19 ± 1	15 ± 1	9 ± 0.5
	F	24 ± 3	17 ± 2	14 ± 1	10 ± 0.5
Phospholipids	M	77 ± 5*	48 ± 1*	26 ± 1	9 ± 0.3
	F	92 ± 4	55 ± 3	25 ± 1	10 ± 0.6
Soluble protein[a]	M	267 ± 19**	167 ± 11**	59 ± 3	1.1 ± 0.2
	F	353 ± 21	211 ± 8	54 ± 3	0.9 ± 0.1
ApoB[a]	M	96 ± 10	85 ± 5	90 ± 4	94 ± 5
	F	91 ± 17	90 ± 9	92 ± 5	95 ± 4

[a] Expressed as number of amino acids using an average residue weight of 100.
Sex differences were tested and, when present, are indicated as in Table 2.

tion, has been discussed in detail by Lindgren et al. (4). The technique is, however, based upon certain assumptions concerning densities, volumes, etc. of the VLDL particles in the calculations of g-minutes needed for the flotation of the different VLDL subclasses. Furthermore the calculation of the diameters of the lipoprotein particles and the ensuing computations of other physicochemical parameters are based upon several other assumptions, particularly concerning the structure of the lipoprotein particle as discussed in some detail by Sata, Havel, and Jones (2) and by Redgrave and Carlson (5). Nevertheless the calculated values for diameters corresponded well to what was predicted for the VLDL subfractions. The LDL particle is spherical with a reported diameter of 17–25 nm and a mass of between 1.8 and 4 million daltons (17). Our calculated values for LDL gave a diameter of 23 nm and a molecular weight of 4 million daltons, in agreement with the values cited above. These facts lend support both to the validity of the technique as such and to the theoretical assumptions underlying the calculations. Furthermore we have, in a few cases, also estimated the size of VLDL subfractions by two other independent techniques, analytical ultracentrifugation and electron microscopy (5). The values for diameters obtained by our calculations agreed well with those obtained with the two physical methods. The calculation of the apparent molecular weight is, however, particularly sensitive to small changes in the assumed value for the thickness of the surface shell. If this shell would be 2.05 nm instead of the assumed value of 2.15, the calculated molecular weight would be 3.3.

Constituents may be lost from lipoprotein particles during isolation by ultracentrifugation as well as by other methods. Ultracentrifugation through a gradient in a swing-out bucket rotor seems more gentle than the repeated centrifugations and washings needed to get uncontaminated fractions when VLDL subfractions are isolated by centrifugation at fixed density in an angle head rotor. The data in **Table 6** show that VLDL subfractions obtained by density gradient ultracentrifugation (present study) and by gel filtration have similar compositions; these data suggest that extensive losses do not occur by ultracentrifugation in density gradients. Furthermore, if losses of material, e.g., protein, occurred in our isolation procedure, they must be very reproducible as there was a rather small variation in the per-

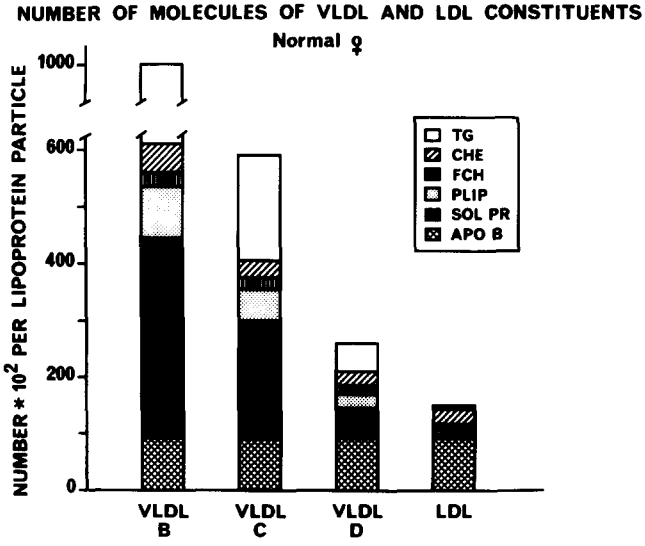

Fig. 3. Number of molecules per lipoprotein particle of the constituents of female VLDL-B, VLDL-C, VLDL-D, and LDL. Abbreviations, see Fig. 2.

TABLE 6. Comparison of the average data obtained in this work for the percentage composition of VLDL-B, VLDL-C, and VLDL-D with the same data for VLDL fractions III, IV, and VI from ref. (2)[a]

Fraction	Sum[b] Pr + Pl + Fc	Protein	Free Chol.	Ester Chol.	P-lipid	Triglycerides	Diameter,[c] nm
B	26.4	9.9	1.7	7.6	14.8	66.1	52.8
III	25.0	7.6	4.5	7.6	12.9	67.3	50
C	31.5	12.0	2.7	9.0	16.8	59.5	42.2
IV	30.5	9.5	5.1	9.4	15.9	60.2	40
D	39.9	14.7	5.8	18.2	19.4	41.9	31.5
VI	39.7	14.5	6.1	15.7	18.7	45.2	30

[a] Fractions III, IV and VI were chosen because they had the closest value for the sum of protein, phospholipids, and free cholesterol as, respectively, VLDL-B, VLDL-C, and VLDL-D. Hence they should correspond in size.
[b] Sum of percentage for protein, phospholipids, and free cholesterol.
[c] The values for fractions III, IV, and VI are very approximate as they are taken from Fig. 8, ref. (2).

centage composition of the constituents. The similar percentage recovery of VLDL triglycerides and cholesterol indicates that there was neither a preferential loss of any of these compounds nor specifically of any of the VLDL subclasses, as these have different cholesterol/triglyceride ratios. For LDL the recovery of cholesterol was similar to that of VLDL, about 90%. LDL triglycerides, however, were recovered only to an extent of 50%. The loss of LDL triglyceride is due to the fact that LDL was sampled in the density region 1.025–1.045 kg/l. Therefore the triglyceride-rich part of LDL with d 1.006–1.019 kg/l (LDL_1, IDL) was not collected. The density region for LDL also explains why Lp(a) or sinking pre-β lipoprotein is excluded from our LDL fraction (see Fig. 1).

The only data on composition of VLDL subclasses from normal subjects that can readily be compared to ours are those from the four to five normolipemic subjects reported by Sata, Havel, and Jones (2). In Table 6 we have listed the data from the three VLDL fractions they obtained by gel chromatography which are closest to our VLDL-B, VLDL-C, and VLDL-D fractions with regard to size. There is in general a good agreement between the percentage composition of the three lipoprotein classes from the two laboratories. The greatest discrepancy is for free cholesterol in the two largest fractions where we found less free cholesterol. Thus we found that 56–70% of the cholesterol was in the esterified form; in corresponding fractions Sata et al. (2) had only 50%. Differences in methodology for the determination of these small amounts of free cholesterol is one possible explanation for these discrepancies. However, the different separation methods may also have played a role. As an example, we (18) and others (19) have found that VLDL isolated by gradient ultracentrifugation in a swinging bucket rotor contains less soluble protein and more cholesterol than VLDL separated by ultracentrifugation in a fixed angle rotor in uniform density.

Large VLDL are transformed into smaller VLDL through the action of lipoprotein lipase. The particles are then further catabolized to still smaller VLDL and eventually to LDL (3). Apart from losses of triglycerides, a larger particle becoming smaller must lose part of the polar surface, consisting of apolipoproteins, free cholesterol, and phospholipids. How do the compositional data reported in this paper fit into this picture? Inspection of Table 5 and Fig. 3 reveals that going from the largest VLDL via the smaller particles to LDL, the particles at each "step" lose molecules of triglycerides, soluble proteins, free cholesterol, and phospholipids, which is compatible with the VLDL catabolism discussed above. Furthermore, the number of molecules of apoB remained constant through all lipoprotein classes indicating that this structural protein remains in the particles during their catabolism. A constant content of apoB in human large and small VLDL as well as in LDL from one patient has been reported by Eisenberg et al. (20). From these data the hypothesis of one VLDL particle becoming one LDL particle was formulated (17).

For cholesteryl esters the picture was different. From VLDL-B to VLDL-C there was a highly significant decrease in the number of molecules, but from VLDL-C to VLDL-D to LDL the number of cholesteryl esters molecules remained unchanged. Thus, not only apoB but also cholesteryl ester molecules remain in the lipoprotein particle when a medium-sized VLDL is transformed to a smaller one and eventually to an LDL particle. This hypothesis is, however, at variance with a recent suggestion of Deckelbaum, Olivecrona, and Eisenberg (21). They believe, based upon data from one hyperlipidemic patient, that VLDL has twice as many cholesteryl ester molecules as LDL (20). Therefore, to get rid of the "excess" cholesteryl ester in the final step of LDL formation, they postulate the presence of a special mechanism involving cholesteryl ester–triglyceride exchange (21). Our data do, however, suggest a much simpler model for the VLDL-to-LDL cascade in normolipidemic subjects which requires only hydrolysis of the triglyc-

erides and loss of redundant surface material. The disappearance of cholesteryl esters from larger to smaller VLDL might be due to a cholesteryl ester–triglyceride exchange (21). or, perhaps more likely, to the disappearance from plasma of some special species of cholesteryl ester-rich large VLDL present in this fraction, e.g., cholesterol-rich remnants (22). The latter possibility is strongly supported by our previous data (5) showing that VLDL-A in hypertriglyceridemia has many more cholesteryl ester molecules than VLDL-B and that VLDL-A during alimentary lipemia in both normo- and hypertriglyceridemic subjects becomes greatly enriched in cholesteryl esters. In fact, a VLDL-A particle had about 20–30 times more cholesteryl ester molecules than a VLDL-B particle (5). These remnant particles must have been removed by the liver and not transformed into VLDL-B.

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