# Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man

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**Abstract** Four subfractions of plasma VLDL characterized by decreasing $S_f$ value and LDL were isolated by density gradient preparative ultracentrifugation from normotriglyceridemic (NTG) and hypertriglyceridemic (HTG) (type IV) subjects in the fasting state and after a fatty meal. Chemical analysis and computation of numbers of particles in each fraction showed that the hyperlipidemia of type IV subjects was accounted for by an increase in total numbers of VLDL and a shift in the distribution of VLDL towards particles of larger diameter. Postprandial hyperlipidemia was due to the presence of chylomicron remnants rather than intact chylomicrons, and was accounted for by an increase in particle diameter of the largest VLDL subfraction rather than by an increase in particle numbers. Postprandial hyperlipidemia was accompanied by a shift in the distribution of VLDL towards particles of larger diameter in both NTG and HTG subjects, probably because of competition for the triglyceride-depletion process between chylomicrons and hepatic VLDL. Most chylomicron remnants were removed from the circulation without degradation to smaller VLDL or to LDL, but some remnants were sufficiently small to contribute to smaller VLDL subfractions. The LDL of type IV subjects contained more apoprotein B than those from NTG subjects, and this difference was associated with increases in diameter, molecular weight, density, and the ratio of protein:phospholipid in LDL from type IV subjects. Defective degradation of large VLDL to small VLDL, and of VLDL to LDL may be related to this alteration in apoprotein B content of the lipoproteins in type IV subjects.—**Redgrave, T. G., and L. A. Carlson.** Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. *J. Lipid Res.* 1979. **20:** 217–229.

**Supplementary keywords** apoprotein B · chylomicrons · chylomicron remnants · plasma triglycerides

Absorption of a fatty meal adds triglyceride-rich intestinal particles to the plasma lipoprotein pool, but postprandial hyperlipidemia is usually of modest proportions (1–3) despite considerable fluxes of absorbed dietary lipids through the plasma compartment. Removal rates of chylomicron triglyceride and cholesterol from the plasma are very rapid in man and in experimental animals, but are reduced in hypertriglyceridemia (3–6), raising the possibility that chylomicrons or their catabolic remnants may persist in the plasma and contribute to hyperlipidemia. Evidence that such a mechanism contributes to hyperlipidemia has been obtained in the cholesterol-fed rabbit (7), in diabetic and hypothyroid rats (8), and in obese and alcohol-fed rats (5, 6).

The present study had two objectives. The first was to define the nature of postprandial hyperlipidemia in terms of lipoprotein number, size, and molecular composition by analyzing the chemical composition of VLDL subfractions. The postprandial changes in normo (NTG)- and hypertriglyceridemic (HTG) subjects were compared to establish if chylomicron remnants contribute to the plasma VLDL pool. Second, we were interested in comparing the composition of VLDL subfractions and of LDL from subjects with type IV HTG with those from NTG individuals.

## MATERIALS AND METHODS

### Subjects and procedures

Seven NTG and seven HTG adult men or women were studied. The NTG subjects had normal blood lipids except for one healthy man classified as having type II A (9) with plasma cholesterol of 7.8 mmol/l (302 mg/dl). The HTG subjects were all classified as having type IV hyperlipoproteinemia (9). Healthy volunteers or patients who had previous myocardial infarction were chosen for the study, excluding

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein: NTG, normotriglyceridemic; HTG, hypertriglyceridemic; EDTA, ethylene diamine tetraacetic acid; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; TG, triglyceride.

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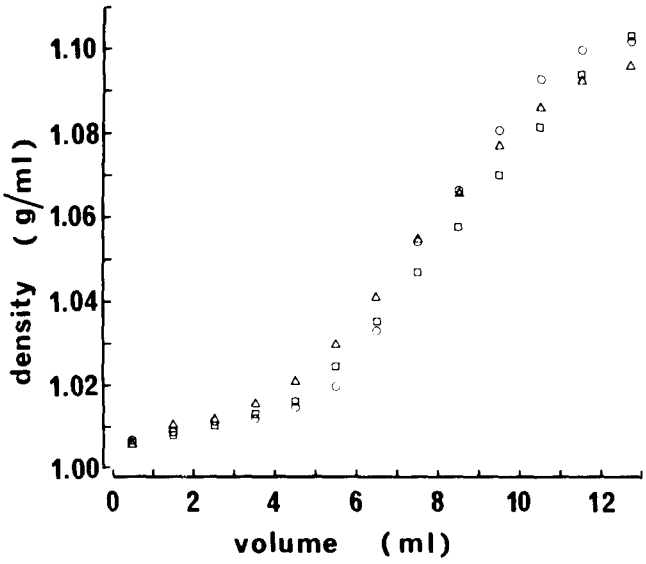


**Fig. 1.** Shape of the density gradient. The densities at 20°C of 1-ml fractions from the centrifuge tube were measured. Points are means of duplicate centrifugations. ○—Not centrifuged, stood on bench for 18 hr; △—centrifuged 28,300 rpm for 43 min (spin A); □—spin A plus 37,000 rpm for 18 hr (spin D).

subjects with either diabetes or other known metabolic disease, or under treatment likely to affect lipid metabolism.

A blood sample was taken on the morning of the test after an overnight fast. Subjects then consumed a test meal of 100 g of soybean oil seasoned with lemon juice. The oil contained 10.8% palmitic, 3.6% stearic, 21.4% oleic, 54.8% linoleic, and 8.9% linolenic acids, giving an average molecular weight for triglycerides of 872. The test meal was tolerated well and no subject was nauseated. Additional blood samples were taken after 3 and 6 hr. Blood was taken into tubes containing 1.4 mg/ml EDTA. Plasma was separated as soon as possible by centrifugation for 20 min at 1000 *g* at room temperature (20–23°C), placed into tubes containing 0.8 mg/ml of *p*-chloromercuriphenylsulfonic acid (10), and stored at 4°C for not longer than 48 hr before lipoprotein separation.

## Methods

A density gradient ultracentrifugation procedure was used to subfractionate the plasma lipoproteins.

Plasma was adjusted to density 1.10 g/ml by adding solid KBr, 0.14 g/ml. Gradients consisting of 4 ml of 1.10 g/ml plasma, 3 ml each of 1.065 g/ml and 1.020 g/ml, and 3.4 ml of 1.006 g/ml salt solutions were formed as described previously (11) and centrifuged in the SW40 rotor of the Beckman L5-75 ultracentrifuge at 20°C.

VLDL was subfractioned by cumulative rate centrifugation, as described by Lindgren, Jensen, and Hatch (12), to float particles of diameter > 75 nm ($S_f > 400$, fraction A), 50–75 nm ($S_f$ 175–400, fraction B), 37–50 nm ($S_f$ 100–175, fraction C), and 20–37 nm ($S_f$ 20–100, fraction D) to the top of the tube. Calculated requirements for these flotations at 20°C were $4.5 \times 10^6$, $17.5 \times 10^6$, $31.2 \times 10^6$, and $152 \times 10^6$ g-min. In the SW 40 rotor, after correction for acceleration and deceleration forces and previous runs, centrifugation was for 43 min at 28,300 rpm (fraction A), then for 67 min at 40,000 rpm (fraction B), then for 71 min at 40,000 rpm (fraction C), and lastly for 18 hr at 37,000 rpm (fraction D). Times given are from switch-on to switch-off of drive power. Maximum acceleration was used, and the brake was not used. Each fraction was carefully aspirated from the top of the tube, and density 1.006 g/ml salt solution was used to refill the tube before the next run. The shape of the gradient is shown in **Fig. 1.** After the final centrifugation, the major LDL fraction was easily identified as a yellow band about 5 mm wide, located between 4 and 5 cm from the bottom of the tube. This band, which contained 67% (SEM 4.0, $n = 7$) of total plasma LDL, was also aspirated from the tube at the end of the procedure.

## Analysis

Triglyceride (13) and cholesterol (14) concentrations in plasma and separated fractions were measured on a Technicon Auto-Analyzer I after lipid extraction (15). Lipid phosphorus was measured (16) after digestion of samples and the values were multiplied by 25 to give phospholipid content. Protein content was determined (17) after extracting with chloroform the turbidity due to lipids. Apoprotein B content of lipoprotein fractions was estimated by difference after extraction of soluble apoproteins with equal volumes of 2-propanol (18) and then 1-pentanol. Extraction with 2-propanol quantitatively precipitates apoprotein B (18) and the subsequent extraction with 1-pentanol removes lipids and produces an aqueous phase concentrated by a factor of 1.48, which greatly facilitates determination of the nonapo-B, soluble apoproteins in the lipoprotein subfractions. High salt concentrations were initially reduced by dialysis to avoid interference with the protein assay.

Esterified and free cholesterol were separated on columns of Sephadex LH-20, modifying the described procedure (19) to employ columns 1 cm in diameter and 20 cm high.

Electrophoresis in agarose gel was according to Noble (20). Disc gel electrophoresis (21) was performed in 8 M urea at pH 9.1 on the peptides soluble after extraction with 2-propanol and 1-pentanol.

Plasma albumin concentrations were measured by rocket immunoelectrophoresis (22) to enable corrections for shifts of body water.

Analytical ultracentrifugation was performed in the Spinco model E, with a 1.2-ml double sector cell in the AN-D rotor. Runs in 1.063 g/ml salt solution at 26°C were at speeds of 10,000 rpm for fraction A, at 20,000 rpm for fractions B and C, at 30,000 rpm for fraction D, and at 40,000 rpm for LDL. F values were corrected to zero concentrations (12) to give the $S_f$ values. Densities were measured at 20°C with a calculating digital density meter (Model DMA 45 Anton Paar KG A-8054 Graz, Austria).

For analysis of the fatty acid composition the VLDL fractions were extracted by chloroform–methanol (15). After separation by thin-layer chromatography (23) the triglyceride fatty acids were methylated and separated on a 5720 A gas chromatograph (Hewlett-Packard) as described (24).

### Calculations

Lipoprotein TG molecular weight was taken to be 860 and that of cholesteryl ester 1.68 × 387. Particle densities were calculated from the partial specific volumes of the constituents; 1.093 for TG; 1.044 for cholesteryl ester; 0.968 for free cholesterol; and 0.970 for phospholipids (25). The partial specific volume for apoprotein is not known, but it was estimated for apoprotein B from the background density of LDL isolated from gradients as described. Background density was measured after ultrafiltration of LDL by membrane filters. Assuming that the LDL band was at its equilibrium density, the partial specific volume of its apoprotein was calculated from measured lipoprotein composition and by employing the known partial specific volumes of the other constituents (Archimedes' principle). A value of 0.7765 ± 0.0030 (SEM, $n = 6$) was obtained and was used for all apoprotein moieties. This value should be compared with those that can be calculated (26) from published (27) amino acid and carbohydrate contents of the apoproteins, viz. 0.731 for apoprotein B; and 0.743, 0.730, and 0.719 for apoproteins C-I, C-II, and C-III, respectively.

The radius, $r_1$, of the VLDL particle was calculated assuming spherical particles with all apolar lipids in the core with radius $r_2$. As the total volume, $V_1$, of all constituents of a VLDL fraction and the volume, $V_2$, of the apolar constituents can be calculated, e.g., per ml in the isolated fractions, the ratio $r_2/r_1 = C = (V_2/V_1)^{1/3}$ can be obtained. Since the data of Sata, Havel, and Jones (25) and Mjøs et al. (28) indicate that all VLDL particles have a constant thickness of the polar surface shell of 2.15 nm it follows that $r_1 - r_2 = 2.15$ and hence $r_1$ was calculated from the relation $r_1 - r_1 \cdot C = 2.15$ or $r_1 = 2.15/(1 - C)$. Particle $S_f$ and molecular weight were calculated assuming Stokes' spheres as described by Lindgren et al. (12) viz. $S_f = D^2 \ (1.063 - d)/1.847$ and $MW = 315.3 \times D^3 \times d$ where $D$ is the diameter in nm and $d$ is the particle density. Particle molecular constituents in daltons were then calculated as the product of weight fraction and molecular weight. Particle numbers were then calculated by dividing the volume of a particle of diameter $D$ into the total lipoprotein volume. All results were corrected for dilution due to KBr solution (4%) and for shifts in body water (0–6%).

## RESULTS

### Characteristics of isolated fractions

The described ultracentrifugal procedure divided plasma VLDL into four subfractions of progressively decreasing diameter, increasing density, and decreasing molecular weight (**Table 1**). Although the calculated molecular parameters of the separated subfractions were within the predicted range of particle dimensions, analytical ultracentrifugation was used to gain an independent assessment of the average size of the fractions. By this method fraction A had diameter 74 nm, fraction B 42.8 nm, fraction C 37.0 nm, fraction D 33.6 nm, and LDL 17.9 nm (means of determinations on two separate samples from fasting HTG subjects), thus confirming the general validity of the calculations, but also showing that calculated diameter is overestimated by 15% on average, perhaps because the calculations assume a uniform population whereas, in fact, each fraction is a continuum. The correlation coefficient between the computed values for diameters obtained by analytical ultracentrifugation and the ratio (volume of apolar constituents)/(total particle volume) was 0.98. Direct measurements by electron microscopy also gave diameters close to the calculated ones, with means of 98 nm for fraction A, 61 nm for fraction B, 46 nm for fraction C, and 35 nm for fraction D (**Fig. 2**).

The material of fraction A did not enter the gel on agarose gel electrophoresis (**Fig. 3**). Fractions B, C, and D all had pre-$\beta$ mobility that was slightly slower postprandial in fraction C and slower in fraction D at all times. Disc-gel electrophoresis in polyacrylamide gels (**Fig. 4**) showed that the proteins soluble after extraction with 2-propanol and 1-pentanol were approximately similar in all fractions, but fraction D con-

TABLE 1. Molecular parameters of lipoprotein fractions

| | NTG | | | HTG | | |
|---|---|---|---|---|---|---|
| | 0 hr | 3 hr | 6 hr | 0 hr | 3 hr | 6 hr |
| | | | *Density g/ml* | | | |
| VLDL | | | | | | |
| A | | 0.932 ± 0.001 | 0.933 ± 0.003 | 0.942 ± 0.002 | 0.933 ± 0.001 | 0.929 ± 0.001 |
| B | 0.954 ± 0.002 | 0.957 ± 0.001 | 0.957 ± 0.001 | 0.955 ± 0.001 | 0.956 ± 0.001 | 0.955 ± 0.002 |
| C | 0.966 ± 0.001 | 0.966 ± 0.001 | 0.967 ± 0.001 | 0.965 ± 0.001 | 0.967 ± 0.001 | 0.970 ± 0.003 |
| D | 0.983 ± 0.002 | 0.984 ± 0.003 | 0.981 ± 0.003 | 0.985 ± 0.002 | 0.984 ± 0.002 | 0.985 ± 0.002 |
| LDL | 1.031 ± 0.002 | 1.032 ± 0.003 | 1.033 ± 0.002 | 1.041 ± 0.003[a] | 1.034 ± 0.000 | 1.038 ± 0.001 |
| | | | *Diameter nm* | | | |
| VLDL | | | | | | |
| A | | 145 ± 13.2 | 159 ± 29.7 | 83 ± 9.8 | 130 ± 5.5[d] | 176 ± 15.5[d] |
| B | 54.4 ± 3.44 | 52.0 ± 1.82 | 50.8 ± 1.85 | 53.8 ± 2.06 | 55.3 ± 1.11 | 54.5 ± 3.93 |
| C | 43.2 ± 0.86 | 44.0 ± 0.71 | 41.6 ± 0.68[c] | 44.2 ± 1.24 | 43.0 ± 2.04 | 40.0 ± 2.12[c] |
| D | 33.0 ± 0.89 | 32.3 ± 1.25 | 33.0 ± 1.30 | 32.4 ± 0.87 | 32.8 ± 1.60 | 31.5 ± 1.32 |
| LDL | 21.8 ± 0.37 | 21.8 ± 0.58 | 21.6 ± 0.24 | 22.5 ± 0.29[b] | 23.0 ± 1.00[b] | 22.5 ± 0.50[b] |
| | | | *Molecular weight* $\times 10^{-6}$ | | | |
| VLDL | | | | | | |
| A | | 935 ± 231.3 | 1678 ± 715.9 | 181 ± 62.0 | 639 ± 76.5[d] | 1698 ± 471.8[d] |
| B | 50.8 ± 9.14 | 43.2 ± 4.26 | 40.6 ± 4.41 | 47.6 ± 5.50 | 50.5 ± 2.90 | 51.0 ± 9.08 |
| C | 25.0 ± 1.64 | 25.5 ± 1.19 | 22.0 ± 1.05[c] | 26.6 ± 2.25 | 24.5 ± 3.23 | 20.3 ± 2.59[c] |
| D | 11.1 ± 0.98 | 10.7 ± 1.17 | 11.5 ± 1.27 | 10.0 ± 1.10 | 11.0 ± 1.49 | 10.1 ± 1.21 |
| LDL | 3.5 ± 0.14 | 3.4 ± 0.20 | 3.3 ± 0.15 | 3.8 ± 0.17[b] | 4.1 ± 0.50[b] | 3.8 ± 0.15[b] |

Results are means ± SEM from five observations in each case except due to sample loss. The fasting sample of fraction A was insufficient for analysis. For each fraction sample 0 hr is fasting sample, 3 hr and 6 hr are times after the fatty meal.

Statistical analysis by Student's $t$ test between NTG and HTG group.

[a] $P < 0.05$.

[b] $P < 0.01$.

Statistical analysis by paired $t$ test to compare pre- and postprandial results within each group.

[c] $P < 0.05$.

[d] $P < 0.01$.

tained relatively more C-I and arginine-rich peptides, and less C-II peptide.

### Lipoproteins in the fasting state, NTG vs. HTG

The triglyceride concentration was higher in plasma and all VLDL fractions in HTG subjects in the fasting state (**Table 2**). All other components of VLDL as well as the number of particles were higher in each VLDL fraction in HTG than NTG subjects (**Tables 2** and **3**). There was a shift towards relatively more larger particles for the HTG group (**Fig. 5**). There was no difference, however, between NTG and HTG subjects with regard to density, diameters, and molecular weights for the four VLDL fractions.

The percentage of apoprotein B content (measured as protein insoluble in 50% 2-propanol plus 1-pentanol) increased progressively from fraction A to fraction D in both NTG and HTG subjects (Table 2). In LDL, 97% (SEM 0.26, $n = 15$) and 96% (SEM 0.53, $n = 7$) of protein was accounted for by apoprotein B in NTG and HTG subjects respectively.

The major LDL band was significantly less ($P < 0.001$) in HTG subjects. Average recovery of total plasma cholesterol in the VLDL subfractions plus the LDL band was 57% in NTG and 67% in HTG subjects. Unaccounted cholesterol in IDL and HDL was not measured in this study.

The molecular mass of all lipoprotein components decreased drastically with decreasing particle diameter except the mass of apoB-peptide (**Table 4**). The far greatest fall was for daltons of triglyceride, from around $10^8$ in VLDL A-fraction to $3 \times 10^5$ in LDL. Table 4 shows that LDL of HTG subjects contained as much phospholipid but more protein than NTG subjects, hence the mean ratio of LDL protein:phospholipid was >1 in the HTG group, and <1 in the NTG group (**Fig. 6,** right-hand side). The major LDL fraction from HTG subjects was more dense and slightly larger than in NTG subjects. The mean molecular weight of LDL of HTG subjects was 14% greater than in NTG subjects. This difference was statistically significant ($P < 0.01$) when observations at all times were pooled.

The individual values for apoprotein B complement

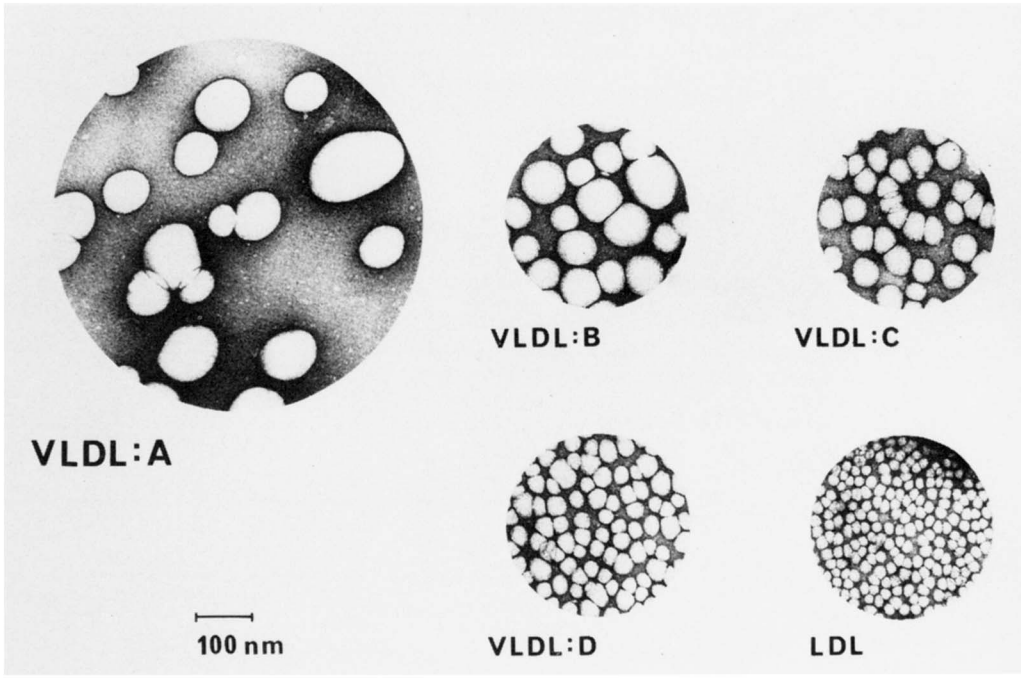


**Fig. 2.** Electron microscopic photographs of the VLDL lipoprotein subfractions and of LDL from a HTG subject at 0 hr. Microscope magnification ×48,000. Measurements of particle diameter of the visualized VLDL fractions gave the following values (mean and range, nm) *A*, 98 (66–159); *B*, 61 (41–97); *C*, 46 (35–62); *D*, 35 (24–45).

in VLDL fractions B, C, and D are plotted in Fig. 6. While there is no obvious difference in apoprotein B content of VLDL from NTG or HTG subjects, it should be noted that the overall mean of the data in Table 4 for NTG subjects is $0.66 \times 10^6$ daltons of apoprotein B, compared with $0.74 \times 10^6$ daltons in HTG subjects. For LDL, however, there is clearly more apoprotein B in HTG subjects viz. 0.89 (SEM 0.03, $n = 8$) $\times 10^6$ daltons compared with 0.65 (SEM 0.01, $n = 15$) for NTG subjects. This difference is highly significant ($P < 0.001$) by Student's $t$ test).

## Effects of the fatty meal

Table 2 shows the changes produced in plasma lipids and in the subfractions after the fatty meal. The postprandial increase in plasma TG was accounted for mainly by the increase in TG of fraction A, but fractions B and C showed small but significant increases in NTG subjects. The rise in triglyceride concentration of fraction A was due to an increase in the size of the particles, not of the numbers. The volume increased about 10 times for HTG subjects (Table 1). It is note-

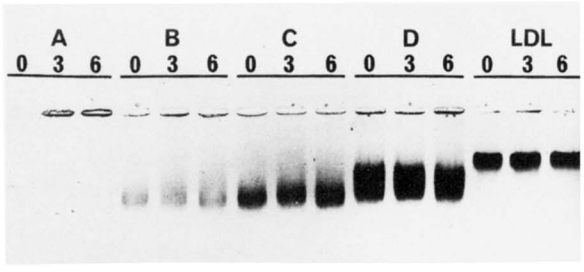


**Fig. 3.** Agarose gel electrophoresis of VLDL subfractions and LDL from a NTG subject. Fraction A does not enter the gel. Fractions B, C, and D show pre-$\beta$ mobility, slightly slower in fraction C and slower in fraction D at all times. 0, 3 hr, and 6 hr indicate elapsed time after fatty meal.

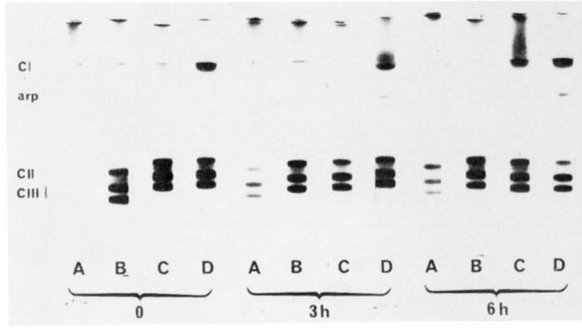


**Fig. 4.** Polyacrylamide disc-gel electrophoresis of VLDL subfractions from a HTG subject. The soluble apoproteins of fraction *D* contain more C-I and arginine-rich peptides, and less C-II peptide postprandially. 0, 3 hr, and 6 hr indicate elapsed time after fatty meal.

TABLE 2. Lipid and protein contents of plasma and lipoprotein fractions

| | NTG | | | HTG | | |
|---|---|---|---|---|---|---|
| | 0 hr | 3 hr | 6 hr | 0 hr | 3 hr | 6 hr |
| Triglyceride concentration ($\mu$mol/ml) | | | | | | |
| VLDL | | | | | | |
| Plasma | 1.70 ± 0.16 | 2.0 ± 0.20 | 2.4 ± 0.21[b] | 5.4 ± 1.06 | 5.8 ± 1.03 | 6.8 ± 1.08[b] |
| A | 0.04 ± 0.01 | 0.24 ± 0.08 | 0.50 ± 0.10[b] | 0.30 ± 0.12 | 0.53 ± 0.15 | 1.69 ± 0.36[b] |
| B | 0.23 ± 0.04 | 0.23 ± 0.04 | 0.33 ± 0.07[a] | 1.70 ± 0.41 | 1.76 ± 0.41 | 1.94 ± 0.38 |
| C | 0.26 ± 0.03 | 0.29 ± 0.02 | 0.32 ± 0.04[a] | 1.33 ± 0.27 | 1.40 ± 0.25 | 1.35 ± 0.22 |
| D | 0.44 ± 0.05 | 0.44 ± 0.05 | 0.46 ± 0.05 | 1.19 ± 0.18 | 1.20 ± 0.13 | 1.04 ± 0.15 |
| Cholesterol concentration ($\mu$mol/ml) | | | | | | |
| VLDL | | | | | | |
| Plasma | 6.4 ± 0.23 | 6.5 ± 0.17 | 6.5 ± 0.19 | 6.5 ± 0.27 | 6.7 ± 0.34 | 6.7 ± 0.22 |
| A | 0.01 ± 0.003 | 0.05 ± 0.02 | 0.08 ± 0.02[b] | 0.08 ± 0.03 | 0.11 ± 0.05 | 0.17 ± 0.04[b] |
| B | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.11 ± 0.02[a] | 0.51 ± 0.14 | 0.50 ± 0.13 | 0.55 ± 0.13 |
| C | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.13 ± 0.02[a] | 0.59 ± 0.15 | 0.57 ± 0.12 | 0.54 ± 0.09 |
| D | 0.36 ± 0.05 | 0.35 ± 0.05 | 0.36 ± 0.04 | 0.96 ± 0.16 | 0.93 ± 0.13 | 0.82 ± 0.12 |
| LDL | 3.09 ± 0.24 | 3.15 ± 0.31 | 3.15 ± 0.35 | 2.12 ± 0.09 | 2.19 ± 0.04 | 2.06 ± 0.17 |
| Phospholipid concentration ($\mu$mol/ml) | | | | | | |
| VLDL | | | | | | |
| A | | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.06 | 0.08 | 0.13 ± 0.03 |
| B | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.07 ± 0.02 | 0.38 ± 0.16 | 0.38 ± 0.16 | 0.51 ± 0.19 |
| C | 0.09 ± 0.02 | 0.10 ± 0.01 | 0.11 ± 0.02 | 0.42 ± 0.14 | 0.43 ± 0.14 | 0.43 ± 0.08 |
| D | 0.23 ± 0.04 | 0.25 ± 0.05 | 0.26 ± 0.03 | 0.70 ± 0.13 | 0.64 ± 0.10 | 0.55 ± 0.11[a] |
| Protein ($\mu$g/ml) | | | | | | |
| VLDL | | | | | | |
| A | 1.3 ± 0.3 | 4.6 ± 1.4 | 7.9 ± 1.4[b] | 7.1 ± 2.4 | 13.9 ± 4.3 | 25.3 ± 5.3[b] |
| B | 16.5 ± 3.1 | 17.5 ± 3.0 | 24.0 ± 4.5[a] | 127.9 ± 29.2 | 127.9 ± 24.7 | 145.9 ± 28.7 |
| C | 29.2 ± 3.5 | 33.1 ± 2.8 | 34.8 ± 4.2[a] | 152.5 ± 29.6 | 160.7 ± 25.5 | 158.0 ± 17.5 |
| D | 91.7 ± 13.0 | 92.7 ± 13.6 | 91.9 ± 9.4 | 249.8 ± 35.8 | 243.4 ± 25.3 | 209.7 ± 24.9 |
| B apoprotein (% total protein) | | | | | | |
| VLDL | | | | | | |
| A | 3.4 ± 3.4 | 4.2 ± 4.2 | 2.8 ± 1.83 | 2.8 ± 2.8 | 9.5 ± 1.85 | 10.0 ± 3.49 |
| B | 19.6 ± 5.04 | 20.8 ± 5.46 | 19.0 ± 3.75 | 25.5 ± 1.71 | 26.3 ± 1.19 | 25.5 ± 1.04 |
| C | 35.4 ± 0.93 | 38.8 ± 2.64 | 36.8 ± 3.69 | 36.5 ± 3.23 | 38.3 ± 1.93 | 41.8 ± 2.10 |
| D | 60.8 ± 1.98 | 60.0 ± 1.58 | 57.6 ± 1.86 | 61.8 ± 3.25 | 62.8 ± 3.42 | 61.8 ± 2.87 |

Details and symbols as in Table 1. Plasma results are from seven subjects each. Because TG and PL were assayed as glycerol and phosphorus, molar units are given. Approximate conversion factors assume molecular weights of 860 for TG, and 775 for PL. The average percentages of cholesterol esterified were 73% in fraction A, 53% in fraction B, 54% in fraction C, 58% in fraction D, and 76% in LDL.

Statistical analysis by paired $t$ test to compare pre- and postprandial results within each group.

[a] $P < 0.05$.

[b] $P < 0.01$.

worthy that HTG subjects showed in plasma and fraction A an increase of much greater magnitude than in NTG subjects.

Total plasma cholesterol was not significantly increased in this study, but cholesterol in fraction A showed a marked postprandial increase in NTG and HTG subjects. Cholesterol was also increased by a small amount in fractions B and C in NTG subjects.

Phospholipids of the VLDL subfractions showed changes similar to those for TG and cholesterol, but the increases were not statistically significant. However, in fraction D phospholipid was significantly decreased 6 hr postprandial.

As shown in Table 2, the protein content in the VLDL subfraction showed changes very similar to those for lipoprotein lipid, with changes most pronounced in fraction A. The relative amount of apoprotein C-II was consistently reduced whereas arginine-rich peptide was increased in postprandial samples in fraction D (Fig. 4). Arginine-rich apoprotein was variably present, and was more consistently found in NTG than in HTG subjects. There was no ap-

TABLE 3. Numbers of particles in the lipoprotein subfractions ($\times 10^{-12}$/ml of plasma)

| | NTG | | | HTG | | |
|---|---|---|---|---|---|---|
| | 0 hr | 3 hr | 6 hr | 0 hr | 3 hr | 6 hr |
| VLDL fraction | | | | | | |
| A | | 0.3 ± 0.1 | 0.3 ± 0.1 | 1.5 ± 0.9 | 0.8 ± 0.4 | 0.7 ± 0.2 |
| B | 3.5 ± 1.1 | 3.4 ± 0.7 | 5.5 ± 1.6 | 24.7 ± 9.7 | 24.4 ± 10.2 | 39.2 ± 20.8 |
| C | 9.4 ± 1.7 | 10.8 ± 1.3 | 13.0 ± 2.3 | 42.1 ± 13.8 | 52.5 ± 22.6 | 52.5 ± 7.9 |
| D | 65.1 ± 28.6 | 55.9 ± 15.5 | 51.9 ± 10.6 | 154 ± 35.8 | 143 ± 32.1 | 127 ± 32.0[a] |
| LDL | 595 ± 29.9 | 645 ± 38.4 | 641 ± 49.5 | 463 ± 30.1 | 395 ± 73.0 | 419 ± 33.5 |

Statistical analysis by paired $t$ test to compare pre- and postprandial results within each group.
[a] $P < 0.05$.

parent qualitative difference between the two groups studied.

Despite the changes observed in the chemical content of the VLDL subfractions after the fatty meal, as shown in Table 3, the calculated numbers of lipoprotein particles in fractions B and C did not change significantly, although a tendency for increased numbers was apparent. In fraction D, numbers were significantly reduced 6 hr after the fatty meal in HTG subjects.

Table 4 shows that particles in fraction A have a much greater molecular mass postprandially for all lipid constituents, in parallel with the observed increase in particle dimensions, but other fractions were not changed by the fatty meal. Unlike all other constituents the mean molecular mass of apoprotein B was remarkably constant in the different fractions. Fraction A appeared to contain more apoprotein B in HTG subjects, but because of the difficulty of measuring the very small amounts of protein in fraction A this figure may be unreliable, although a similar high value was observed in rat large lipoproteins (28).

The relative amounts in VLDL triglycerides of the two most characteristic fatty acids of the fatty meal, linoleic (18:2) and linolenic (18:3) acid, present in 54.8 and 8.9%, respectively, in the fat, are given in **Table 5.** In fraction A the percentage of 18:2 and 18:3 increased 2- to 3-fold postprandially in both NTG and HTG subjects. At 6 hr the relative content of 18:2 and 18:3 in fraction A had approached the corresponding values of the fatty meal. The relative amounts of these fatty acids rose also in the other VLDL fractions but to a lesser degree and the percentage figures for 18:2 and 18:3 did not reach one-fourth of that for the ingested fat. Furthermore, the rise was smaller the smaller the VLDL particles were.

While the increase in relative amount of 18:2 and 18:3 was similar in fraction A for NTG and HTG subjects, it was smaller for HTG than NTG individuals in the VLDL fractions B to D. This difference became more pronounced the smaller the VLDL. For instance, in VLDL fraction D the percentage of 18:3 rose by about 1% for NTG but only by 0.1% for HTG subjects.

## DISCUSSION

Our findings confirm and extend many previous studies of postprandial hyperlipidemia after a fatty meal (1–3). A more complete quantitative description of postprandial hyperlipidemia than before has been obtained by our subfractionation and chemical characterization of the lipoproteins. In particular our experimental design permitted observations of the possible contribution of chylomicrons and their remnants to smaller diameter fractions of the plasma lipo-

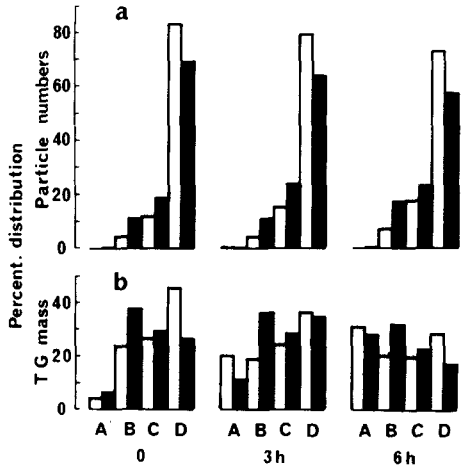


**Fig. 5.** Percentage distribution of particle numbers (*a*) and TG mass (*b*) in VLDL subfractions. □ NTG, ■ HTG (data from Tables 2 and 3). Compared with NTG subjects, HTG subjects show a relative shift towards particles of larger size. After a fatty meal, both groups show a shift towards larger particles, without an increase in total particle number. The mass of TG is distributed more evenly than particle numbers, but similar differences between NTG and HTG subjects are apparent, as are changes induced by the fatty meal.

TABLE 4. Molecular mass of lipoprotein constituents (daltons × $10^{-6}$)

| | NTG | | | HTG | | |
|---|---|---|---|---|---|---|
| | 0 hr | 3 hr | 6 hr | 0 hr | 3 hr | 6 hr |
| Triglyceride | | | | | | |
| VLDL | | | | | | |
| A | | 754 ± 211.3 | 1444 ± 642.1 | 133 ± 51.0 | 526 ± 73.0 | 1484 ± 435.6 |
| B | 35.2 ± 7.14 | 28.8 ± 3.38 | 26.2 ± 3.35 | 32.0 ± 4.44 | 34.5 ± 2.50 | 34.8 ± 6.86 |
| C | 15.0 ± 1.22 | 15.8 ± 0.95 | 12.8 ± 0.73 | 16.0 ± 1.70 | 15.0 ± 2.38 | 11.9 ± 2.02 |
| D | 5.3 ± 0.65 | 5.0 ± 0.74 | 5.5 ± 0.80 | 4.5 ± 0.67 | 5.1 ± 0.90 | 4.5 ± 0.71 |
| LDL | 0.2 ± 0.04 | 0.2 ± 0.02 | 0.2 ± 0.01 | 0.3 ± 0.05 | 0.3 ± 0.09 | 0.3 ± 0.11 |
| Cholesteryl ester | | | | | | |
| VLDL | | | | | | |
| A | | 91 ± 21.0 | 110 ± 37.0 | 19 ± 5.7 | 42 ± 2.5 | 80 ± 11.6 |
| B | 3.4 ± 0.60 | 3.5 ± 0.25 | 3.6 ± 0.18 | 3.9 ± 0.30 | 3.7 ± 0.17 | 3.8 ± 0.68 |
| C | 2.3 ± 0.11 | 2.3 ± 0.05 | 2.2 ± 0.04 | 2.7 ± 0.13 | 2.4 ± 0.30 | 1.9 ± 0.22 |
| D | 1.7 ± 0.06 | 1.6 ± 0.10 | 1.7 ± 0.11 | 1.6 ± 0.10 | 1.8 ± 0.18 | 1.6 ± 0.15 |
| LDL | 1.5 ± 0.09 | 1.4 ± 0.13 | 1.4 ± 0.10 | 1.6 ± 0.11 | 1.8 ± 0.45 | 1.5 ± 0.20 |
| Free cholesterol | | | | | | |
| VLDL | | | | | | |
| A | | 20 ± 4.7 | 24 ± 8.2 | 4 ± 1.3 | 9 ± 0.5 | 18 ± 2.5 |
| B | 1.8 ± 0.32 | 1.9 ± 0.14 | 1.9 ± 0.10 | 2.0 ± 0.17 | 1.9 ± 0.09 | 2.0 ± 0.36 |
| C | 1.1 ± 0.07 | 1.2 ± 0.03 | 1.1 ± 0.02 | 1.4 ± 0.06 | 1.2 ± 0.15 | 1.0 ± 0.10 |
| D | 0.7 ± 0.03 | 0.7 ± 0.04 | 0.7 ± 0.05 | 0.7 ± 0.05 | 0.8 ± 0.07 | 0.7 ± 0.06 |
| LDL | 0.3 ± 0.02 | 0.3 ± 0.03 | 0.3 ± 0.03 | 0.3 ± 0.04 | 0.3 ± 0.02 | 0.3 ± 0.06 |
| Phospholipid | | | | | | |
| VLDL | | | | | | |
| A | | 53 ± 11.0 | 76 ± 24.5 | 20 ± 4.2 | 48 ± 4.0 | 90 ± 16.5 |
| B | 7.6 ± 0.87 | 6.6 ± 0.49 | 6.3 ± 0.51 | 7.0 ± 0.53 | 7.2 ± 0.31 | 7.3 ± 0.83 |
| C | 4.4 ± 0.22 | 4.5 ± 0.17 | 4.0 ± 0.18 | 4.4 ± 0.22 | 4.2 ± 0.34 | 3.8 ± 0.43 |
| D | 2.3 ± 0.17 | 2.3 ± 0.19 | 2.4 ± 0.23 | 2.1 ± 0.15 | 2.2 ± 0.19 | 2.1 ± 0.17 |
| LDL | 0.8 ± 0.05 | 0.8 ± 0.07 | 0.8 ± 0.04 | 0.8 ± 0.04 | 0.8 ± 0.07 | 0.8 ± 0.01 |
| Soluble apoprotein | | | | | | |
| VLDL | | | | | | |
| A | | 17 ± 4.8 | 23 ± 9.0 | 4 ± 2.0 | 13 ± 2.6 | 25 ± 5.7 |
| B | 2.2 ± 0.43 | 2.1 ± 0.17 | 1.9 ± 0.18 | 2.1 ± 0.33 | 2.4 ± 0.30 | 2.3 ± 0.40 |
| C | 1.3 ± 0.08 | 1.2 ± 0.02 | 1.1 ± 0.04 | 1.3 ± 0.14 | 1.3 ± 0.20 | 1.0 ± 0.13 |
| D | 0.5 ± 0.03 | 0.5 ± 0.03 | 0.5 ± 0.04 | 0.5 ± 0.07 | 0.5 ± 0.09 | 0.4 ± 0.08 |
| LDL | 0.03 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.00 |
| Apoprotein B | | | | | | |
| VLDL | | | | | | |
| A | | | 0.76 ± 0.51 | | 1.04 ± 0.13 | 2.74 ± 1.07 |
| B | 0.54 ± 0.18 | 0.60 ± 0.16 | 0.59 ± 0.05 | 0.67 ± 0.10 | 0.86 ± 0.11 | 0.77 ± 0.14 |
| C | 0.70 ± 0.04 | 0.79 ± 0.07 | 0.65 ± 0.09 | 0.75 ± 0.13 | 0.78 ± 0.10 | 0.72 ± 0.10 |
| D | 0.73 ± 0.05 | 0.69 ± 0.07 | 0.66 ± 0.05 | 0.64 ± 0.06 | 0.75 ± 0.08 | 0.68 ± 0.04 |
| LDL | 0.67 ± 0.02 | 0.64 ± 0.02 | 0.64 ± 0.02 | 0.90 ± 0.06[a] | 0.88 ± 0.10 | 0.86 ± 0.01 |

[a] $P < 0.01$, comparison by Student's $t$ test.

proteins, and of differences in chylomicron clearances between NTG and HTG subjects.

The data of Tables 2, 3, and 4 show that almost all of postprandial hyperlipidemia is accounted for by increases in mass in fraction A.

Despite the 'chylomicronemia' the increase in chemical constituents of fraction A is accounted for by an increase in particle size (Tables 1 and 4), not particle numbers. The small numbers of particles in fraction A during fat absorption requires explanation. However it can readily be calculated that 100 g of fat produces only $2.6 \times 10^{16}$ chylomicrons of diameter 200 nm. This represents an influx of $3.6 \times 10^{10}$ min$^{-1}$ ml$^{-1}$ of plasma if the fat load is assumed to be absorbed uniformly over 4 hr. If chylomicron t½ = 5 min in NTG subjects (3, 4, 29) then turnover is 0.14 min$^{-1}$, and the calculated influx rate corresponds to a steady-state concentration of $0.26 \times 10^{12}$ particles/ml,

which agrees with our observations (Table 3). Similarly in HTG subjects if chylomicron t½ = 15 min the steady-state plasma content can be calculated to be $0.78 \times 10^{12}$ particles/ml, again in accord with our observations (Table 3). Chemical composition of fraction A shows that in NTG subjects the 'chylomicron' fraction contains about 10% cholesteryl ester, which is more consistent with a TG-depleted chylomicron remnant than a chylomicron. Remnants still are sufficiently large to float in this fraction (30). In HTG subjects, fasting fraction A also has a high cholesteryl ester content, but postprandial samples show less TG depletion than in NTG subjects. Nevertheless, fraction A analysis shows considerably more cholesteryl ester than the 1.4% of human lymph chylomicrons (31). The high proportion of cholesterol esterified (73%) in fraction A is consistent with its origin from intestinal lipoproteins, as is its content of linoleic and linolenic acids (Table 5) which in postprandial samples approached that of the ingested fat both in NTG and HTG subjects. Apoproteins A are present in chylomicrons from lymph (31) but were not observed in fraction A of postprandial samples so are presumably lost when chylomicrons are degraded to remnants.

The increases in mass of lipoprotein in fractions B and C in NTG subjects are accompanied by small increases in particle numbers of these fractions, but the changes are not statistically significant. In fact fraction D shows a decrease in particle numbers, which cancels possible increases in numbers of the smaller fractions so that total numbers of lipoprotein particles do not increase in the total VLDL (Table 3). However,

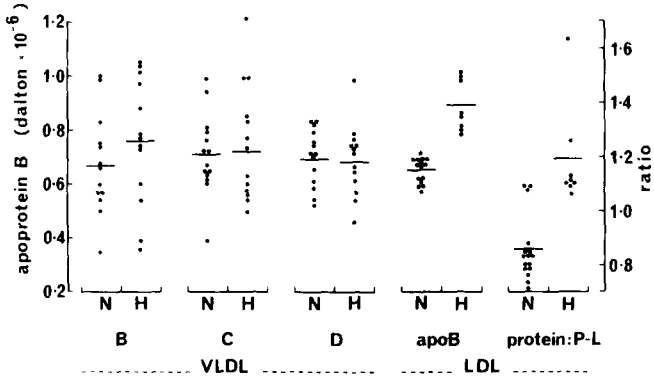


**Fig. 6.** Apoprotein B molecular complement and protein: phospholipid ratio. Individual values for apoprotein B complement of VLDL fractions B, C, and D and LDL are shown, with the means (bar). The LDL from HTG subjects contain about one-third more than LDL from NTG subjects but, because variability is greater, VLDL subfractions show no difference in content of apoprotein B between NTG and HTG subjects. The LDL protein:phospholipid ratio in HTG is greater than in most NTG subjects. Note that the right-hand axis refers only to the protein:phospholipid ratio on the extreme right-hand side of the figure.

TABLE 5. Content of linoleic (18:2) and linolenic (18:3) acid in the triglycerides of the four VLDL fractions and of LDL from two subjects in each group

| | NTG | | HTG | |
|---|---|---|---|---|
| | 0 hr | 6 hr | 0 hr | 6 hr |
| Linoleic acid, % | | | | |
| VLDL fraction | | | | |
| A | | 51.3 | 14.5 | 40.3 |
| | | 45.7 | 11.0 | 49.8 |
| B | 21.0 | 38.4 | 6.74 | 10.5 |
| | 19.2 | 36.4 | 13.0 | 21.6 |
| C | 19.1 | 33.9 | 7.13 | 8.37 |
| | 18.8 | 32.7 | 13.2 | 18.8 |
| D | 18.1 | 27.1 | 6.24 | 7.44 |
| | 18.1 | 28.3 | 11.7 | 16.5 |
| LDL | 16.6 | 18.8 | 10.2 | |
| | 13.1 | 17.8 | 12.3 | 14.2 |
| Linolenic acid, % | | | | |
| VLDL fraction | | | | |
| A | | 6.22 | 2.45 | 5.10 |
| | | 5.97 | 1.20 | 6.35 |
| B | 2.45 | 4.26 | 0.65 | 1.10 |
| | 1.64 | 4.04 | 0.77 | 2.19 |
| C | 1.60 | 3.70 | 0.47 | 0.79 |
| | 0.89 | 2.98 | 1.44 | 1.90 |
| D | 1.36 | 2.43 | 0.52 | 0.62 |
| | 0.65 | 2.08 | 1.44 | 1.66 |
| LDL | 1.37 | 1.41 | 1.5 | |
| | 0.72 | 1.04 | 0.64 | 0.94 |

as shown in Fig. 5*a*, the distribution of particles is different between HTG and NTG subjects, and the difference is accentuated after a fatty meal. Both groups show a shift in the population towards larger particles after the fatty meal but this is not reflected in individual subfractions (Table 1).

There is convincing evidence that the smaller VLDL particles (fraction D) are derived by triglyceride depletion from larger VLDL (32). The different shapes of the populations in the fasting state suggest that VLDL depletion is less efficient in HTG subjects compared with controls (Fig. 5*a*). When the catabolic load on the lipolytic pathway is increased after the fatty meal, both NTG and HTG subjects show a shift in the population towards larger sizes, most likely as a consequence of competition for lipolysis between chylomicrons and large VLDL (33). By comparing the distribution of lipoprotein numbers and triglyceride mass (Fig. 5*a* and *b*), it is seen that hypertriglyceridemia in HTG subjects is due to a shift in the population toward larger particles, together with an increase in particle numbers (Table 3).

Two discontinuities are apparent in the otherwise

smooth decline of the molecular masses of constituents (except apoprotein B) as one proceeds from larger to smaller particles (Table 4). The first discontinuity is in postprandial samples between fraction A and fraction B, indicating that fraction A might not be progressively depleted of constituents until it attains the molecular proportions of fraction B, but instead might be removed directly from the plasma while still retaining the molecular characteristics of fraction A. This interpretation is consistent with removal of chylomicron remnants by the liver as in the rat (30) when only 80–90% of particle triglyceride has been removed.

On the other hand the data of Table 5 show clear increases in the proportions of linoleic (18:2) and linolenic (18:3) acid in the smaller VLDL fractions, such that newly ingested triglyceride constitutes about one-third of fractions B and C in NTG subjects, but less than one-tenth of these fractions in HTG subjects. Smaller proportions appear in fraction D. Three different mechanisms could account for this observation. First, chylomicron remnants might be degraded sufficiently to appear in these fractions (vide infra). Second, chylomicron remnant triglyceride fatty acids might be resecreted by the liver after their initial clearance. Third, constituent fatty acids released by the action of lipoprotein lipase in peripheral tissues might be recycled as hepatic VLDL within the time course of the experiment. The relative significance of these mechanisms is unknown.

The second discontinuity occurs between fraction D, the smallest fraction of VLDL, and LDL and is most apparent for TG, phospholipid, and soluble apoprotein (Table 4). It is a consequence of the catabolic conversion of VLDL to LDL, through IDL. Although IDL was not studied in these experiments it has a chemical composition between that of fraction D and LDL (34–36).

The molecular mass of apoprotein B within each fraction of VLDL (excluding fraction A) plus LDL is remarkably constant (Table 4). This finding extends those of Hammond and Fisher (37), Mjøs et al. (28), and Eisenberg and Rachmilewitz (36). In all cases, VLDL and LDL particles contain about $0.7 \times 10^6$ daltons of apoprotein B, and this value is similar in rat and man. Our study confirms this finding for VLDL and LDL in man. Values of $0.3–0.5 \times 10^6$ reported for human VLDL by Eisenberg et al. (38) appear too low, in part, apparently, because of incorrect assumptions about particle hydrated density used to calculate molecular weight. For example, a particle of $S_f$ 147 was assumed to have a hydrated density of 0.94 g/ml, but our measurements indicate a hydrated density of 0.96 g/ml.

Our data allow a comparison of apoprotein B content between NTG and HTG groups. There is a small but significant difference between the groups for LDL but not for VLDL. Nevertheless, on average VLDL from HTG subjects contain $0.74 \times 10^6$ daltons of apoprotein B compared with $0.66 \times 10^6$ daltons in NTG subjects. For LDL however, HTG subjects contain $0.89 \times 10^6$ daltons of apoprotein B compared with a mean of $0.65 \times 10^6$ daltons for the NTG group. Our data should be compared with values of $0.74–0.88 \times 10^6$ daltons for LDL from type IV subjects and $0.63–0.64 \times 10^6$ daltons for LDL from normal or type II subjects reported by Hammond and Fisher (37) and Fisher, Hammond, and Warmke (39). Our data confirm the difference in LDL composition, for which one possible explanation could be the addition of eight monomeric units of apoprotein B, which has a reported molecular weight of 27,500 (40). Hence LDL from NTG subjects would contain 24 monomeric units of apoprotein B ($0.66 \times 10^6$ daltons) whereas LDL from HTG subjects may contain 32 monomeric units ($0.88 \times 10^6$ daltons). Also plausible is the possibility that LDL from NTG subjects contains three subunits of apoprotein B with a polypeptide chain of $0.22 \times 10^6$ daltons, whereas LDL from HTG subjects contains four such subunits. This possibility agrees well with the size of the polypeptide chain reported by Simons and Helenius (41) and Smith, Dawson, and Tanford (42). Our data suggest, but do not prove, that VLDL from HTG subjects also contains more apoprotein B than VLDL from NTG subjects, which would be consistent with the generally accepted concept that LDL particles are derived from VLDL particles without loss of this peptide.

Table 1 shows that the principal LDL band from HTG subjects also differs in other ways. It has a slightly higher hydrated density, larger molecular diameter, and greater molecular weight than LDL from NTG subjects. Because of the increase in protein content the ratio of protein to phospholipid is reversed (Fig. 6) independently of any assumptions that could overestimate LDL molecular weight. The relationship established between surface and core constituents for larger particles might not be completely applicable to LDL although recently a spherical model for LDL has been proposed, with a thickness of the polar surface shell of 2.13 nm (43). Furthermore our calculations ignore particle hydration (44) and assume uniform spherical particles. Nevertheless calculated LDL molecular weight is in fair agreement with recent hydrodynamic measurements by Fisher et al. (39, 44) ($2.4–3.5 \times 10^6$) and Nelson et al. (45) ($2.9 \times 10^6$) using sedimentation equilibrium. Hydrated density found by these workers agrees well with our measure-

ments. If LDL molecular weight were somewhat overestimated by our calculations, comparisons between NTG and HTG subjects would not be invalidated. The content of apoprotein B appears to be greater in HTG particles compared with NTG controls. This difference may underlie the defective catabolism in HTG subjects of VLDL to smaller particles that is apparent in our results. It may also explain the smaller numbers of LDL particles found in HTG subjects (Table 3). Furthermore, such a change in the catabolism of VLDL may be related to our previous finding that there are differences in the surface material of VLDL subclasses between NTG and HTG so that HTG VLDL have a reduced ratio apo C-II/apo C-III (46). It will be of interest to know if the differences in LDL composition and in apoprotein B content persist after treatments of HTG subjects with lipid-lowering regimes (47).

Our conclusions concerning the catabolism of chylomicrons should be compared with those of Hazzard and Bierman (48) who studied the appearance of radioactive vitamin A in chylomicrons and VLDL ($S_f$ 20–400) 6 hr and 24 hr after a fatty meal. At 6 hr most vitamin A was in the chylomicron fraction, which is consistent with our conclusion that chylomicron remnants contribute largely to fraction A, and are removed from this fraction without extensive degradation to smaller particles. However on the basis of their recovery of most vitamin A radioactivity in $S_f$ 20–400 VLDL after 24 hr, Hazzard and Bierman (48) concluded that chylomicron remnants persisted as small particles in the plasma. Two considerations make this explanation unlikely. First, a chylomicron of diameter 200 nm that contains 1.4% cholesteryl ester (31) contains about $58 \times 10^3$ nm$^3$ of cholesteryl ester, whereas a particle from fraction B (Table 1) with diameter 54 nm has a total volume of only $82 \times 10^3$ nm$^3$. Because the cholesteryl ester content of fraction B is not more than 11% (Tables 2 and 3), particles containing about 70% cholesteryl ester clearly do not contribute significantly to fraction B nor to smaller subfractions. Table 4 shows that the molecular mass of cholesteryl ester increases *only* in fraction A after the fatty meal. The numbers of cholesteryl molecules in the particles can be readily calculated from the data of Table 4. This calculation serves to emphasize the different character of the particles in fraction A, which contain 15- to 80-fold more cholesteryl ester molecules than particles of fraction D or LDL. Particles of fractions B and C contain up to three times more than fraction D or LDL, but still very much less than fraction A. Fractions B and C hence show evidence for only a minor contribution of chylomicron remnants by this criterion, also.

Second, because absorption of triglyceride from the intestine will be completed within 24 hr, chylomicrons or their remnants will not persist in the plasma by virtue of their well-documented brief lifetimes in the circulation. Because of these considerations, the appearance of dietary linoleic and linolenic acid (Table 5) in the smaller VLDL subfractions cannot be taken as unequivocal evidence that chylomicron remnants are catabolized so completely within the vascular compartment. As discussed earlier, resecretion of VLDL by the liver of fatty acids released peripherally or after hepatic uptake of remnant triglyceride could contribute to this observation. The simplest interpretation of our results is that in man, as in the rat (30), most chylomicron remnants are removed rapidly by the liver without appreciably contributing to smaller VLDL subfractions or LDL.

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