# Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey

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**Abstract** The cholesteryl ester content of plasma low density lipoproteins (LDL) in monkeys has previously been shown to be related to the rate of hepatic cholesterol secretion and cholesteryl ester content of newly secreted lipoproteins in the isolated perfused liver. In the present studies, African green monkeys were fed diets containing cholesterol and 40% of calories as either butter or safflower oil in order to determine the effects of saturated versus polyunsaturated dietary fat on hepatic lipoprotein secretion. The rate of cholesterol accumulation in liver perfusates was correlated with the size of the donor's plasma LDL, but for any rate, a smaller plasma LDL was found in donor animals of the safflower oil group than in those of the butter group. Hepatic very low density lipoproteins (VLDL) were smaller in the safflower oil group but contained more cholesteryl ester and fewer triglyceride molecules per particle than those from the butter group. Livers from the safflower oil group contained more cholesteryl ester and less triglyceride than those from the butter group. The cholesteryl ester percentage composition of hepatic VLDL resembled that of the liver in each group. The data show that dietary polyunsaturated fat decreased plasma LDL size even though it increased the cholesteryl ester content of lipoproteins secreted by the liver. Therefore, intravascular formation of plasma LDL from hepatic precursor lipoproteins appears to include the removal of relatively greater amounts of cholesteryl esters from the precursor lipoproteins in polyunsaturated fat-fed animals. —**Johnson, F. L., R. W. St. Clair, and L. L. Rudel.** Effects of the degree of saturation of dietary fat on the hepatic production of lipoproteins in the African green monkey. *J. Lipid Res.* 1985. **26:** 403–417.

**Supplementary key words** liver • cholesterol • polyunsaturated fat • saturated fat • perfusion • secretion • primates

Previous studies have demonstrated that dietary cholesterol affects the size, composition, and concentration of plasma low density lipoproteins (LDL) in several species of nonhuman primates (1, 2), and the change in LDL has been related to the severity of diet-induced coronary artery atherosclerosis (2–5). Dietary cholesterol increases plasma LDL size by increasing the number of cholesteryl ester molecules per LDL particle (1). Using the isolated perfused liver of African green monkeys, we have demonstrated that dietary cholesterol causes increased hepatic secretion of cholesteryl esters among a large spectrum of lipoprotein types (6). Although none of the nascent lipoproteins of the liver perfusate resembled plasma LDL in composition or structure, many did have the same densities as LDL. The rate of hepatic cholesterol secretion in the isolated liver system was correlated to the size of the plasma LDL from the liver donor animal. It seems likely that most of the normal and cholesteryl ester-enriched plasma LDL are derived from postsecretory modifications of at least some of the hepatic lipoproteins after the precursor particles have entered the circulation in vivo.

In related studies in African green monkeys we found that, relative to polyunsaturated fat, dietary saturated fat increases plasma LDL size and cholesteryl ester content as well as the degree of saturation of plasma LDL cholesteryl esters (7). We hypothesized that dietary fat saturation would, in turn, affect the degree of saturation and amount of cholesteryl ester secreted in lipoproteins by the liver. In the present studies, this hypothesis was tested in African green monkeys fed diets containing 40% of calories as either butter fat or safflower oil. The isolated livers from these animals were perfused by recirculation with a lipoprotein-free medium in order to study the effects of these dietary fats on hepatic lipoprotein production.

## MATERIALS AND METHODS

### Animals and diets

Adult male African green monkeys (*Cercopithecus aethiops*) of the vervet subspecies, weighing 3.3–5.9 kg, were purchased from an animal importer (Primate Imports,

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin: cholesterol acyltransferase.



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Port Washington, NY). Two groups were established based on the degree of fat saturation in the diet. The diets fed to the animals have been described previously (see 75-8B and 75-8S diets, ref. 8). Each diet contained 0.78 mg cholesterol/kcal and 40% of calories as either butter fat or safflower oil. The animals were fed their diets for 2 to 5 years before livers were taken for perfusion. The animals were monitored monthly for total serum and HDL cholesterol concentrations. Routine clinical monitoring included blood urea nitrogen, hemoglobin, hematocrit, and differential cell count. All animals remained in good health throughout the study as evidenced by the fact that body weights remained constant and were comparable between diet groups.

### Perfusion of livers

Animals were fed 10-14 hr prior to the liver perfusion. Animals were anesthetized with ketamine hydrochloride (Bristol Laboratories, Div. Bristol-Myers, Syracuse, NY) 10-20 mg/kg. Liver perfusion was performed essentially as described previously (6) except that Tygon tubing 3/32" i.d. × 5/32" o.d. (Norton Co., Akron, OH) was used for the portal vein cannula.

The perfusion medium was Krebs-Henseleit original Ringer bicarbonate containing glucose, amino acids, insulin, cortisol, penicillin, and streptomycin as described previously (6). Hydrocortisone (Sigma Chemical Co., St. Louis, MO) at 250 ng/ml medium was substituted for hydrocortisone sodium succinate used previously, and the medium was replenished with 125 ng/ml every 30 min during the perfusion. Washed human erythrocytes from outdated blood were included in the medium at a hematocrit of 22%.

To minimize contamination from plasma lipoproteins, perfusion by recirculation was performed for 90 min in order to allow the liver to release plasma lipoproteins, after which the perfusate was replaced with fresh medium. Perfusion by recirculation was then continued for 4 hr, and lipoproteins that had accumulated in the perfusate during this period were characterized. Perfusate entering and leaving the liver was sampled at 30-min intervals throughout the experiments to monitor $PO_2$, $PCO_2$, and cholesterol concentration. In each case, oxygen consumption remained essentially constant during perfusion, indicating good viability of the livers that were used.

### Lipoprotein isolation and fractionation

At the completion of the experiment the perfusate was collected on ice and made to final concentrations of 0.04% 5,5'-dithiobis-(2-nitrobenzoic acid), 0.1% EDTA, 0.1% $NaN_3$, pH 7.4. Erythrocytes were removed by centrifugation. All solutions subsequently used for lipoprotein isolation contained 0.01% EDTA, 0.02% $NaN_3$, pH 7.4. Very low density lipoproteins (VLDL) were isolated from the perfusate by ultracentrifugation for 18 hr at 53,000 rpm ($3.1 \times 10^8$ *g*-min) at 4°C in a 60Ti rotor (Beckman Instruments, Inc., Cedar Grove, NJ). The tubes were sliced, and the top fraction containing VLDL was removed. This centrifugation also pelleted erythrocyte ghosts from the perfusate. VLDL were washed and concentrated by recentrifugation under the same conditions. The density of the infranatant from the first spin was raised to 1.225 g/ml with solid KBr, and perfusate lipoproteins of density 1.006-1.225 g/ml were isolated as above by ultracentrifugation for 40 hr ($6.9 \times 10^8$ *g*-min). The tubes were sliced and the top fraction containing the lipoproteins was washed and concentrated by recentrifugation under the same conditions.

Isolated VLDL were fractionated by gel filtration chromatography on a 1.5 × 90 cm column of 2% agarose (Bio-Gel A-50m, 100-200 mesh, Bio-Rad Laboratories, Richmond, CA) eluted with 0.9% NaCl, 0.01% EDTA, 0.02% $NaN_3$, pH 7.4. Perfusate lipoproteins of density 1.006-1.225 g/ml were fractionated on a column of 4% agarose (Bio-Gel A-15m, 200-400 mesh, Bio-Rad) into size populations referred to as regions I, II, III, and IV (see Fig. 3) as described previously (6). Material within these size populations was then pooled, concentrated, and subfractionated by density gradient ultracentrifugation as described previously (6). Density subfractions were dialyzed exhaustively against 0.01% EDTA, 0.02% $NaN_3$, pH 7.4, before further analysis.

### Electron microscopy

Lipoproteins were observed by negative stain electron microscopy with 2% potassium phosphotungstate, pH 6.5, as described previously (6) using a Philips TEM 400 electron microscope equipped with a high tilt goniometer stage (Philips Electronic Instruments, Inc., Mahwah, NJ). To obtain a final concentration of approximately 2 mg/ml, some samples were concentrated directly on Formvar carbon-coated grids by evaporation under a stream of nitrogen. Diameters of VLDL fractions were measured from photographs with a total magnification of 147,000× using a sonic digitizer (Science Accessories Corp., Southport, CT). At least 200 particles per fraction were measured. Exact magnifications were determined from a calibration grid photographed during each microscopic session.

### Analytical methods

Plasma LDL molecular weights were determined by gel filtration chromatography as described previously (9). Lipids were extracted from livers and lipoproteins according to the method of Folch, Lees, and Sloane Stanley (10). Cholesterol content was measured according to the method of Rudel and Morris (11). Lipid phosphorus content was measured according to the method of Fiske and Subbarow (12). Protein content was estimated according to Lowry et al. (13), extracting with hexane to



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remove turbidity after color development. Bovine serum albumin Fraction V (Sigma Chemical Co.) was used as the reference standard. Thin-layer chromatography of lipoprotein and liver lipids was performed essentially as described previously (6). Triglyceride was measured according to the method of Sardesai and Manning (14). Cholesteryl esters were fractionated and quantitated according to the method of Carroll and Rudel (15). ApoB was measured by electroimmunoassay (16) using antisera, raised in goats, to apoB purified from cynomolgus monkey plasma LDL.

Apoproteins from isolated lipoproteins were separated by electrophoresis in a horizontal slab, linear gradient of 4 to 30% polyacrylamide, and a 4% polyacrylamide stacking gel was also used. Buffers were those used by Laemmli (17) and contained 0.1% sodium dodecyl sulfate (SDS). Lipoprotein samples were lyophilized and then resolubilized in 0.46% barbital, 5% mercaptoethanol, 3% SDS, 0.001% bromphenol blue, pH 8.3, at 100°C for 2 min.

### Calculations

The volume of VLDL particles was calculated from diameters measured by electron microscopy assuming a spherical structure. The volume of the core of VLDL particles was calculated assuming a constant shell thickness of 21.45 Å obtained from the data of Sata, Havel, and Jones (18). Cholesteryl ester and triglyceride were assumed to be contained solely in the core of the particles and to be the only core constituents. Partial specific volumes of the constituents were taken to be (in ml/g): triglyceride, 1.093; cholesteryl ester, 1.044; free cholesterol, 0.968; phospholipids, 0.970; and protein, 0.705 (18).

### Statistical analysis

Values in text, tables, and figures are expressed as the arithmetic mean ± standard error of mean. Statistical comparisons were performed using the two-tailed Student's *t*-test.

## RESULTS

### Plasma lipoprotein responses to diet

Animals fed the butter diet had higher average concentrations of serum cholesterol, LDL cholesterol, plasma apoprotein B, and HDL cholesterol, and had higher average LDL molecular weights than animals receiving the safflower oil diet (**Table 1**).

### Accumulation of liver perfusate cholesterol

The rate of accumulation of liver perfusate total cholesterol was essentially linear during the perfusion (**Fig. 1**) and was not different between diet groups when normalized for liver weight, averaging 2.73 ± 0.2 mg/hr per 100 g of liver for the butter group and 2.70 ± 0.4 mg/hr per 100 g of liver for the safflower oil group. The rate of accumulation of perfusate cholesterol was correlated with the donor animal's plasma LDL molecular weight in both diet groups ($r$ = 0.69 for butter-fed animals, and $r$ = 0.80 for safflower oil-fed animals, **Fig. 2**). The slopes of the regression lines were not different between diet groups, but the y-intercept was significantly greater in the butter-fed animals than in the safflower oil-fed animals ($P < 0.05$); thus for any LDL molecular weight, the rate of accumulation of cholesterol in the perfusate was greater in safflower oil-fed animals than in butter-fed animals.

### Size distribution of liver perfusate lipoproteins

The size distribution of perfusate lipoproteins was different from that of plasma lipoproteins in both diet groups. The elution profiles from agarose column chromatography of plasma and perfusate lipoproteins from a butter-fed and a safflower oil-fed animal are shown in **Fig. 3.** The profiles shown are from two animals picked to be representative for their diet groups in relative lipoprotein mass distribution. Plasma LDL eluted in the center peak; HDL eluted in the later peak (9). The elution profiles of liver perfusate lipoproteins of d 1.006–1.225 g/ml from the same two animals showed that the perfusate

TABLE 1. Effects of degree of dietary fat saturation on plasma lipoproteins

| Diet | n | Serum Chol. | LDL Chol. | Plasma ApoB[a] | LDL Molecular Weight | HDL Chol. |
|---|---|---|---|---|---|---|
| | | *mg/dl* | *mg/dl* | *mg/dl* | *g/μmol* | *mg/dl* |
| Butter | 12 | 278 ± 37 | 153 ± 30 | 119 ± 21 | 3.76 ± 0.09 | 75 ± 5 |
| Safflower oil | 12 | 174 ± 17[b] | 79 ± 20[b] | 73 ± 11[b] | 3.30 ± 0.13[b] | 63 ± 8[b] |

[a]Butter group, n = 8; safflower oil group, n = 9.
[b]Significantly different from butter group, $P < 0.05$.



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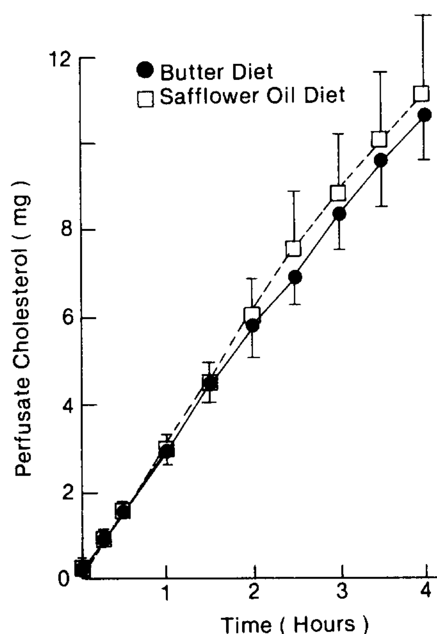


**Fig. 1** Cholesterol accumulation in liver perfusate. Livers from animals fed the butter diet (•) and the safflower oil diet (□) were perfused by recirculation for 1.5 hr followed by a 10-min flush, after which recirculation perfusion was resumed with 300 ml of fresh medium for an additional 4 hr. Three-ml samples of perfusate were taken at the indicated times during this 4-hr period. Erythrocytes and erythrocyte ghosts were removed from the samples by high speed centrifugation, and cholesterol was assayed. Values are the mean of 11 butter-fed animals and 8 safflower oil-fed animals ± standard error of mean.

lipoproteins were typically larger than the plasma lipoproteins. In perfusate lipoproteins of both diet groups there was no eluted peak of material corresponding in size to plasma LDL. The size distributions for perfusate lipoproteins from each diet group were different; a greater amount of region I material and a smaller amount of region III material was consistently found in butter-fed animals compared to safflower oil-fed animals. In both diet groups, perfusate lipoproteins eluting in region IV were heterogeneous in size as indicated by the broadness of the peak (Fig. 3). These lipoproteins have been subfractionated and analyzed in greater detail and will be discussed in a separate report.

**Table 2** summarizes the differences in perfusate lipoprotein distribution in terms of the percentage mass and absolute mass distributions of total cholesterol. Liver perfusates from butter-fed animals contained an average of 3 times as much cholesterol in lipoproteins of size region I, 60% more in lipoproteins of region II, and 45% less in lipoproteins of region III compared to livers from safflower oil-fed animals. These differences between diet groups were observed when the data were expressed on a percentage basis or on a cholesterol mass basis. No difference was observed between diet groups in the amounts of cholesterol in the lipoproteins of region IV.

Fractionation of perfusate VLDL by 2% agarose column chromatography (**Fig. 4**) revealed that perfusate VLDL from butter-fed animals were larger on the average ($K_{av}$ = 0.400 ± 0.017, n = 7) than perfusate VLDL from safflower oil-fed animals ($K_{av}$ = 0.465 ± 0.014, n = 5, $P < 0.05$). The elution peaks of some animals' perfusate VLDL were divided into five fractions (I–V) of equal volumes with fraction III being set as the center of the peak. The small mass of material eluting in the void volume of the column was not included. Measurement of these VLDL fractions by negative stain electron microscopy also indicated that VLDL fractions from livers of butter-fed animals were larger in diameter than VLDL from livers of safflower oil-fed animals (**Table 3**, $P < 0.01$ by analysis of variance).

### Structure of perfusate lipoproteins

Perfusate VLDL and lipoproteins of column regions I, II, and III were structurally heterogeneous. By electron microscopy most perfusate VLDL appeared spherical (round), but some particles appeared to have an appended membrane-like tab or sheet of excess surface material (**Fig. 5**). Lipoproteins from column regions I, II, and III were similar to each other in their overall structural appearance except for the average size decrease proceeding from region I to region III (**Fig. 6**). Many particles within these column regions also had the membrane-like tab; this tab often comprised a very large portion of the particle, especially in regions I and II. The

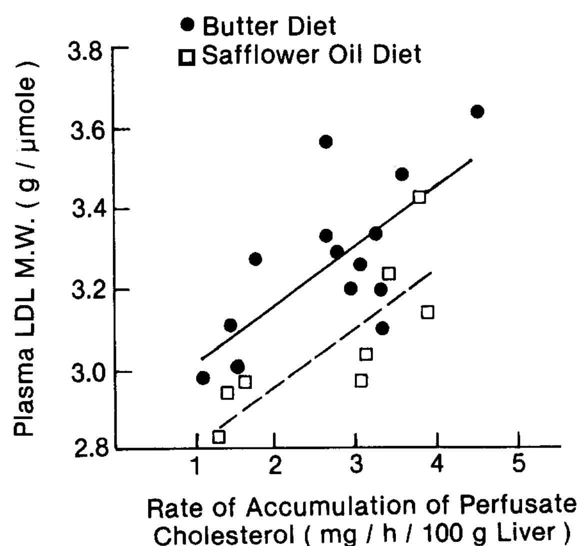


**Fig. 2** Relationship of plasma LDL molecular weight to rate of cholesterol accumulation in perfusates. Plasma LDL molecular weight of the liver donor was determined as previously described (9). The rate of accumulation of perfusate cholesterol was taken as the slope of the least squares linear regression of values of perfusate cholesterol content versus time (Fig. 1), normalized to 100 g of liver.



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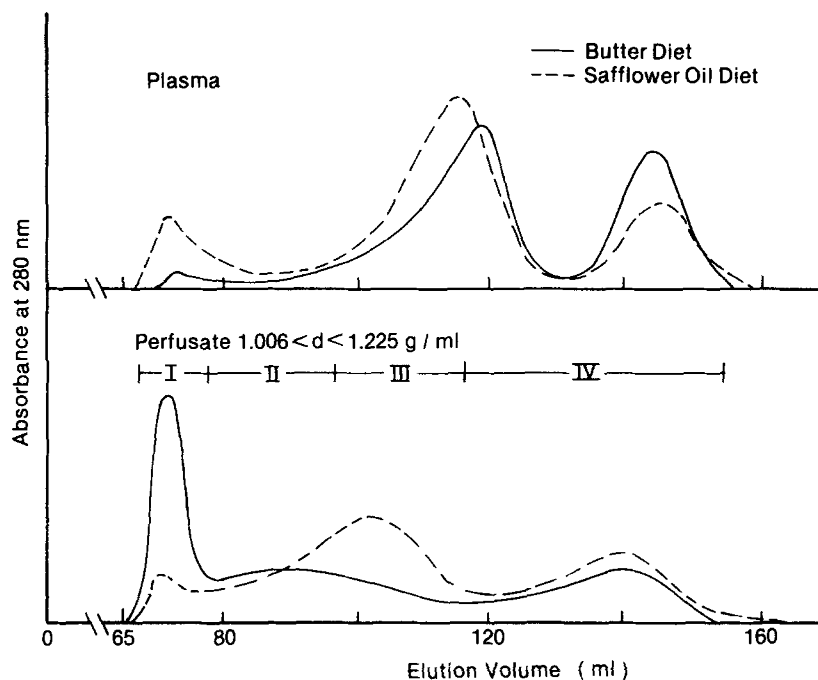


**Fig. 3** Four percent agarose column chromatography of lipoproteins from plasma and liver perfusate. Plasma lipoproteins were isolated initially by ultracentrifugation of plasma at d 1.225 g/ml. Perfusate VLDL were removed from the perfusate by ultracentrifugation at d 1.006 g/ml after which the density of the perfusate was raised to 1.225 g/ml and all other perfusate lipoproteins were isolated by ultracentrifugation. Plasma lipoproteins (upper panel) and d 1.006–1.225 g/ml liver perfusate lipoproteins (lower panel) from an animal fed the butter diet (solid line) and an animal fed the safflower oil diet (broken line) were separated on a column of 4% agarose. The data for perfusate lipoproteins are from the same two animals as those for plasma lipoproteins. The perfusate lipoprotein fractions that were pooled for further analyses are indicated by Roman numerals.

tab appeared to be continuous with a more electron-lucent portion of the particle that is interpreted to be the spherical core of nonpolar lipids located between the leaflets of a membrane bilayer. The tab was often observed on its edge, showing a minimum thickness for this structure of 45–50 Å. Stereo pair electron micrographs (**Fig. 7**) confirmed this generalized structure for perfusate VLDL and lipoproteins in column regions I, II, and III by revealing the three-dimensional relationship between the spherical and tab portions of the particles. The portion of an individual particle that was spherical and electron-lucent varied widely among lipoproteins within any column region; some particles appeared full of core material while others appeared empty, consisting solely of

TABLE 2. Cholesterol distribution among perfusate lipoproteins

| Diet | n | VLDL | Column Fractions[a] | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | I | II[b] | III[b] | II + III[c] | IV | | |
| | | | *percentage* | | | | | | |
| Butter | 11 | 25.5 ± 3.4 | 18.6 ± 2.6 | 18.7 ± 2.6 | 22.0 ± 3.3 | 38.8 ± 2.6 | 15.4 ± 1.3 | | |
| Safflower oil | 7 | 32.5 ± 4.0 | 6.1 ± 1.4 | 11.5 ± 1.5 | 32.7 ± 4.1 | 44.2 ± 6.1 | 17.5 ± 2.7 | | |
| | | | *µg/hr per 100 g of liver* | | | | | | |
| Butter | 9 | 719 ± 134 | 512 ± 81 | 565 ± 138 | 531 ± 97 | 1230 ± 184 | 407 ± 28 | | 2868 ± 298 |
| Safflower oil | 7 | 975 ± 216 | 169 ± 49[d] | 353 ± 56 | 939 ± 189[d] | 1242 ± 252 | 464 ± 55 | | 2849 ± 384 |

[a]Fractions are pooled regions eluted from a 4% agarose column. See Fig. 3 for location of each fraction. Recovery of cholesterol after ultracentrifugal concentration and column chromatography was 88 ± 2%.
[b]Butter group, n = 7; safflower oil group, n = 6.
[c]These values include experiments in which perfusate lipoproteins eluting in column fractions II and III were not taken separately.
[d]Significantly different from butter group, $P < 0.05$.



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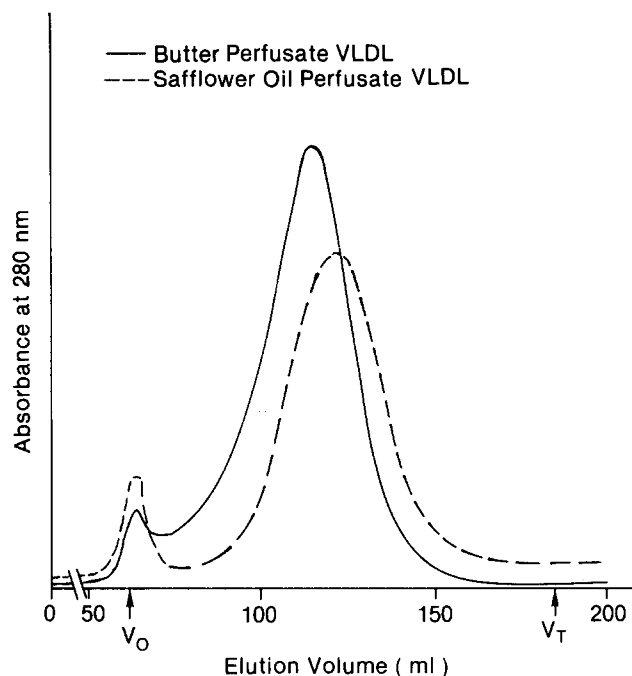


**Fig. 4** Two percent agarose column chromatography of perfusate VLDL. VLDL were isolated from the perfusate of a butter-fed animal (solid line) and a safflower oil-fed animal (broken line) by ultracentrifugation at d 1.006 g/ml and were then applied and eluted from a column of 2% agarose.

a sheet of surface material. In general, lipoproteins of column region I had the most excess surface material whereas lipoproteins of region III had the least. An exception to the general particle structure just described was the presence of amorphous, vesicular-appearing particles found to varying degrees among the lipoproteins of region I.

Lipoproteins of column region IV were high density lipoproteins and consisted largely of discoidal particles having a wide range of sizes (45–190 Å in diameter), many of which tended to form rouleaux upon negative staining (**Fig. 8**). These particles are the subject of a separate publication in preparation in which they will be described in detail.

### Apoprotein composition of liver perfusate lipoproteins

There were no diet-related differences consistently seen in the apoprotein composition of the major perfusate lipoprotein subfractions (**Fig. 9**). VLDL and lipoproteins from column regions I, II, and III had similar apoprotein compositions. The large molecular weight form of apoB (corresponding to that of plasma LDL) was a major apoprotein on these perfusate subfractions. There was no detectable protein band on any perfusate subfraction from either diet group corresponding to the lower molecular weight form of apoB. ApoE was present in all of these lipoproteins to varying degrees, but the ratio of apoE to apoB consistently appeared greatest in column region I, least in region III, and intermediate in region II. VLDL resembled region III in its apoE to apoB ratio. Small amounts of the low molecular weight apoproteins, i.e., serum amyloid A protein (SAA), apoC, and perhaps apoA-II, were also present on perfusate VLDL and column region I, II, and III lipoproteins. An unidentified protein with a molecular weight of about 44,000 was found as a component of column region I to a highly variable degree in both diet groups. This protein was also found in very small amounts in column regions II and III.

### Composition of subfractions of liver perfusate lipoproteins

The perfusate lipoproteins of column regions I, II, and III were further fractionated by density gradient ultracentrifugation to yield more homogeneous subfractions suitable for compositional analysis. Recovery after concentration, density gradient ultracentrifugation, and dialysis was 85 ± 3%. No consistent diet-related differences were observed in the density distribution of cholesterol from these column regions. For column region I, 11 ± 1.5% of the cholesterol was isolated at d < 1.02 g/ml and 83 ± 1.5% was isolated at d 1.02–1.065 g/ml. In regions II and III, 25 ± 2.0% of the cholesterol was isolated at d < 1.02 g/ml, and 70 ± 2.0% was isolated at d 1.02–1.065 g/ml. Two peaks were often evident within the d 1.02–1.065 g/ml range (data not shown) so that subfractions d 1.02–1.03 g/ml and d 1.03–1.065 g/ml were taken

TABLE 3. Size of perfusate VLDL subfractions

| | | VLDL Subfraction[a] | | | | |
|---|---|---|---|---|---|---|
| Diet | n | I | II | III | IV | V |
| | | *diameter (Å)* | | | | |
| Butter | 5 | 438 ± 37 | 433 ± 26 | 351 ± 12 | 346 ± 10 | 345 ± 14 |
| Safflower oil | 4 | 365 ± 26 | 360 ± 32 | 326 ± 14 | 321 ± 10 | 303 ± 12 |

[a]VLDL subfractions were obtained by column chromatography on 2% agarose. The eluted peak of VLDL was divided into five fractions of equal volumes with fraction III representing the center of the eluted peak. Diameters of subfractions were determined by negative stain electron microscopy; at least 200 particles per subfraction were measured.



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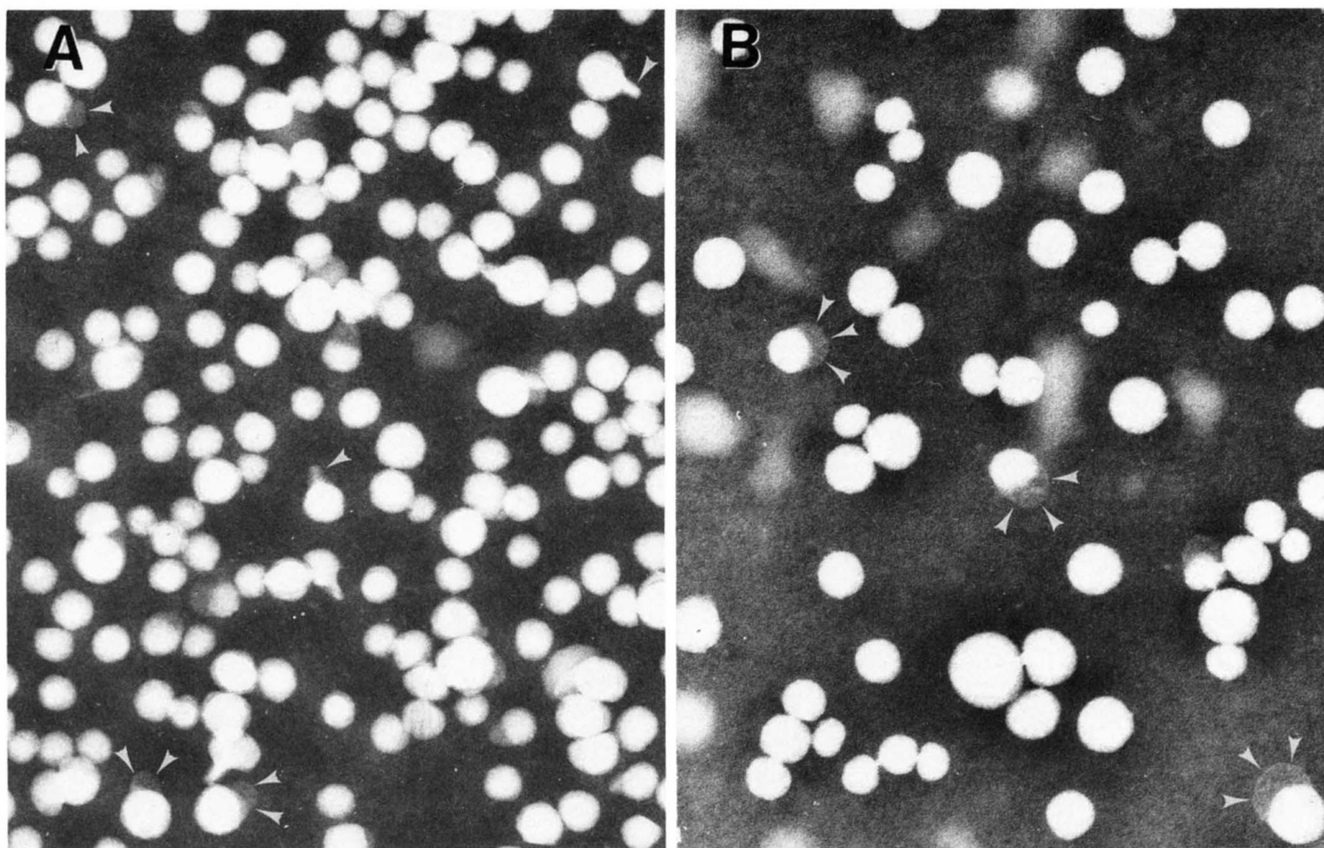


**Fig. 5** Negative stain electron micrographs of liver perfusate VLDL. Perfusate VLDL subfraction III from a butter-fed animal (panel A) and subfraction I from a safflower oil-fed animal (panel B) were negatively stained with 2% potassium phosphotungstate, pH 6.5. Arrows indicate particles with extra surface material in the form of a membrane-like tab. Bar marker indicates 1000 Å.

separately when sufficient material was available for further chemical analysis.

The effects of dietary fat saturation on the chemical composition of the major perfusate lipoprotein subfractions are summarized in **Table 4.** All of the triglyceride-rich perfusate lipoproteins from butter versus safflower oil-fed animals (VLDL, $II_{1.006-1.02}$, and $III_{1.006-1.02}$) were characterized by a lower percentage mass of cholesteryl ester and a higher percentage mass of triglyceride. The mass percentages of other components of these particles were not different between diet groups. The diet-related difference in mass percentages of cholesteryl ester and triglyceride was due to an absolutely higher rate of accumulation of triglyceride and lower rate of accumulation of cholesteryl ester in triglyceride-rich perfusate lipoproteins from butter versus safflower oil-fed animals (**Table 5**).

All of the perfusate lipoproteins with densities between 1.02 g/ml and 1.065 g/ml from both diet groups were especially rich in phospholipid and free cholesterol and were relatively poor in nonpolar lipids (Table 4), consistent with the surface-rich appearance of these particles by electron microscopy (Figs. 6 and 7). Similar to the triglyceride-rich subfractions, the more dense lipoproteins from the butter group were also relatively enriched in triglyceride and were relatively poor in cholesteryl ester compared to the same lipoproteins from the safflower oil group. These diet-related differences were found to be due to absolute differences in the rates of cholesteryl ester and triglyceride accumulation in these subfractions (Table 5), similar to the observations in the triglyceride-rich subfractions.

## Cholesteryl ester content of liver perfusate VLDL

In order to determine whether the diet-related differences in the chemical composition of perfusate lipoproteins were due to absolute differences in lipoprotein cholesteryl ester content, the percentage composition and average particle size of perfusate VLDL subfractions obtained by agarose column chromatography were determined, thus making within-particle calculations possible. Perfusate VLDL from both diet groups were heterogeneous in size (Table 3, Fig. 4). For each animal in both diet groups, the number of molecules of cholesteryl ester per particle was not constant for VLDL subfractions of different sizes (**Fig. 10**). Rather, this number increased in a linear



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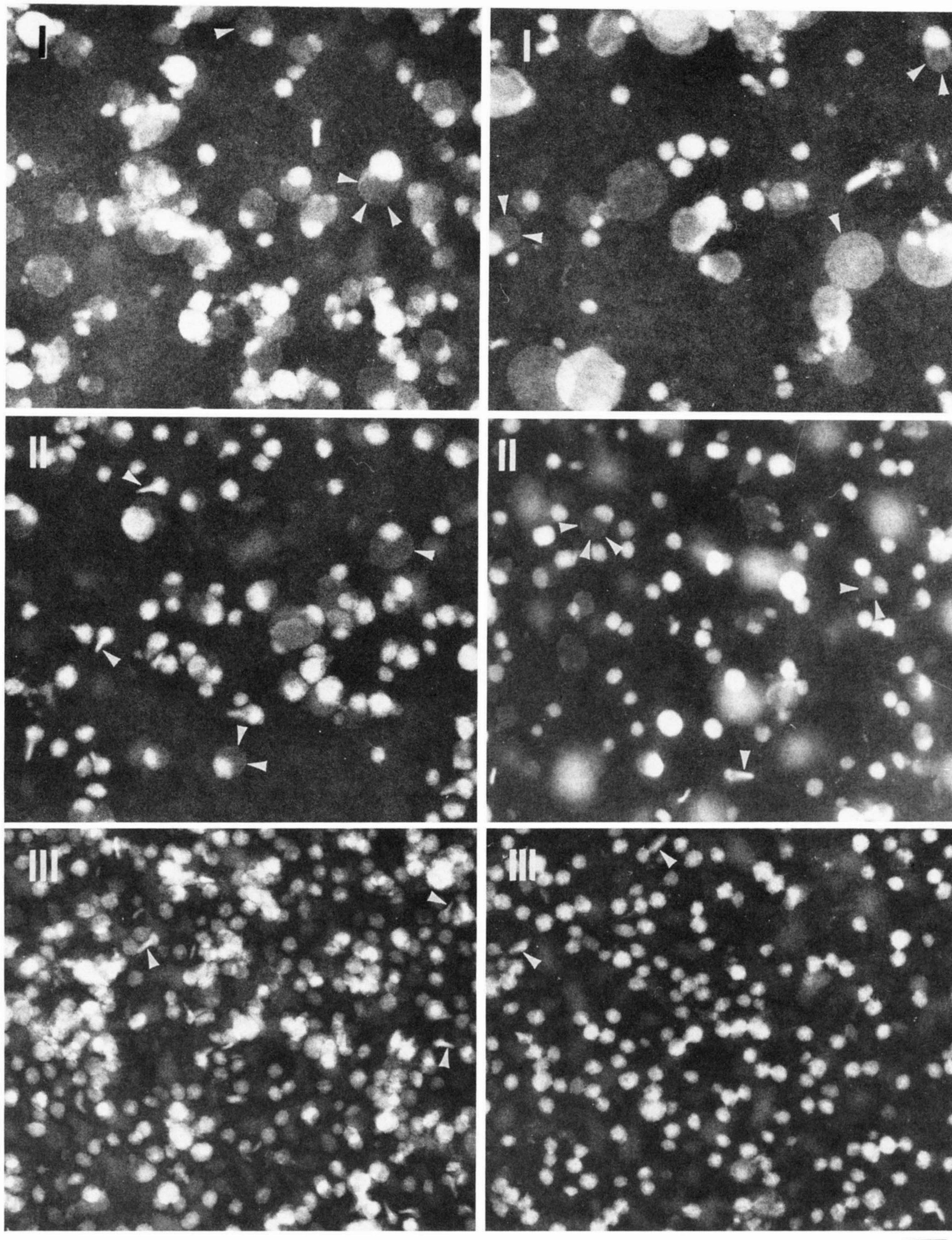




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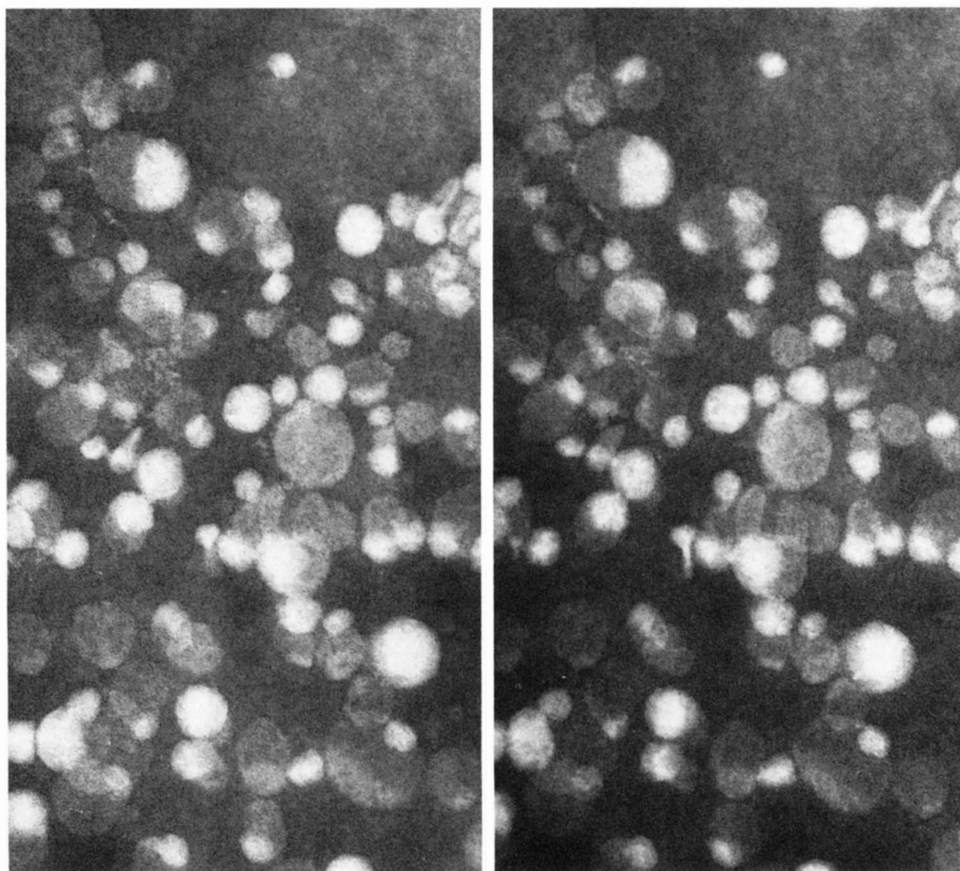

**Fig. 7** Stereo-pair negative stain electron micrograph of perfusate lipoproteins. Perfusate lipoproteins from a butter-fed animal were obtained from region I by agarose column chromatography and were negatively stained. Electron micrographs were made at a magnification of 117,000 × at a tilt angle of ± 18°. Membrane-like tabs can be seen on a large variety of particles containing different amounts of core material. Many particles contain no core material. Particles are apparently embedded throughout a matrix of potassium phosphotungstate and are not spread as a monolayer attached to the Formvar surface. Bar marker indicates 1000 Å.

fashion as the average volume of the particle increased. However, as particle size increased, the accompanying increase in the number of cholesteryl ester molecules contained per particle was greater in perfusate VLDL from safflower oil-fed animals than from butter-fed animals. For any size of VLDL particle, there were more cholesteryl ester molecules and necessarily fewer triglyceride molecules contained per particle in perfusate VLDL from the safflower oil-fed group compared to those from the butter-fed group.

## Liver lipid composition

Histologically, only occasional hepatocytes contained observable lipid accumulation in the form of droplets; biochemically, lipid concentrations were elevated less than twofold over the levels in livers of monkeys fed a low cholesterol, low fat diet (data not shown). However, livers from safflower oil-fed animals had over twice the cholesteryl ester concentration of livers from butter-fed animals (3.77 ± 0.7 vs. 1.62 ± 0.2 mg/g) and only a third of the triglyceride concentration of livers from butter-fed animals (1.01 ± 0.2 vs. 3.15 ± 0.5 mg/g, **Fig. 11**).

## Cholesteryl ester composition of liver and liver perfusate VLDL

The effects of dietary fat saturation on cholesteryl ester composition of liver and liver perfusate VLDL are summarized in **Table 6.** The distribution of cholesteryl esters

**Fig. 6** Negative stain electron micrographs of perfusate lipoproteins. Perfusate lipoproteins isolated by ultracentrifugation between d 1.006 g/ml and 1.225 g/ml were separated by 4% agarose column chromatography as in Fig. 3. Fractions within regions indicated by Roman numerals I–III were pooled and examined by negative stain electron microscopy. Perfusate lipoproteins are from a butter-fed animal (left column) and from a safflower oil-fed animal (right column). Arrows indicate particles with extra surface material in the form of a membrane-like tab occasionally seen on edge. Bar marker indicates 1000 Å.



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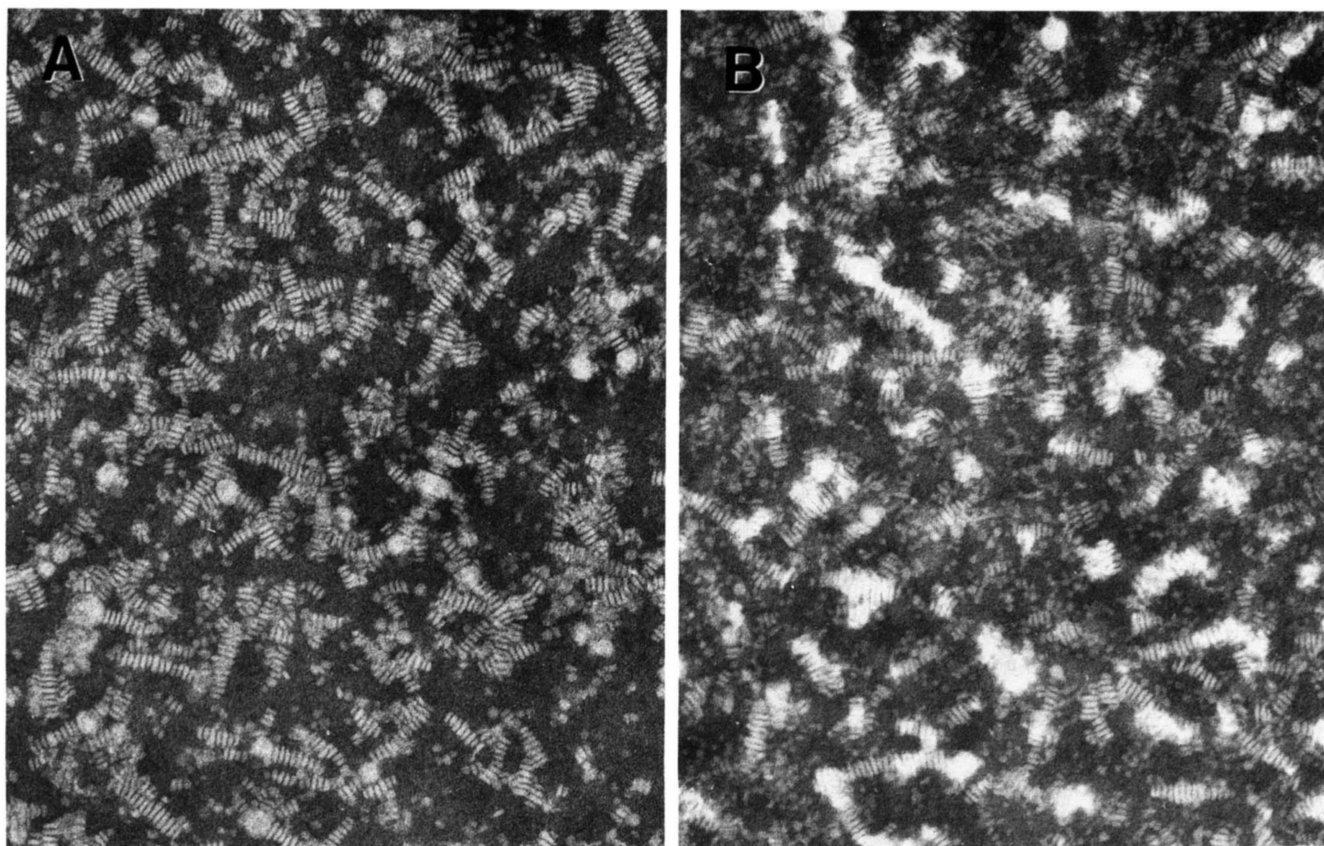


**Fig. 8** Negative stain electron micrographs of liver perfusate HDL. Perfusate HDL were isolated by ultracentrifugation and separated by 4% agarose column chromatography. Fractions within region IV from a butter-fed animal (panel A) and a safflower oil-fed animal (panel B) as shown in Fig. 3 were pooled and negative stain electron microscopy was performed. Bar marker indicates 1000 Å.

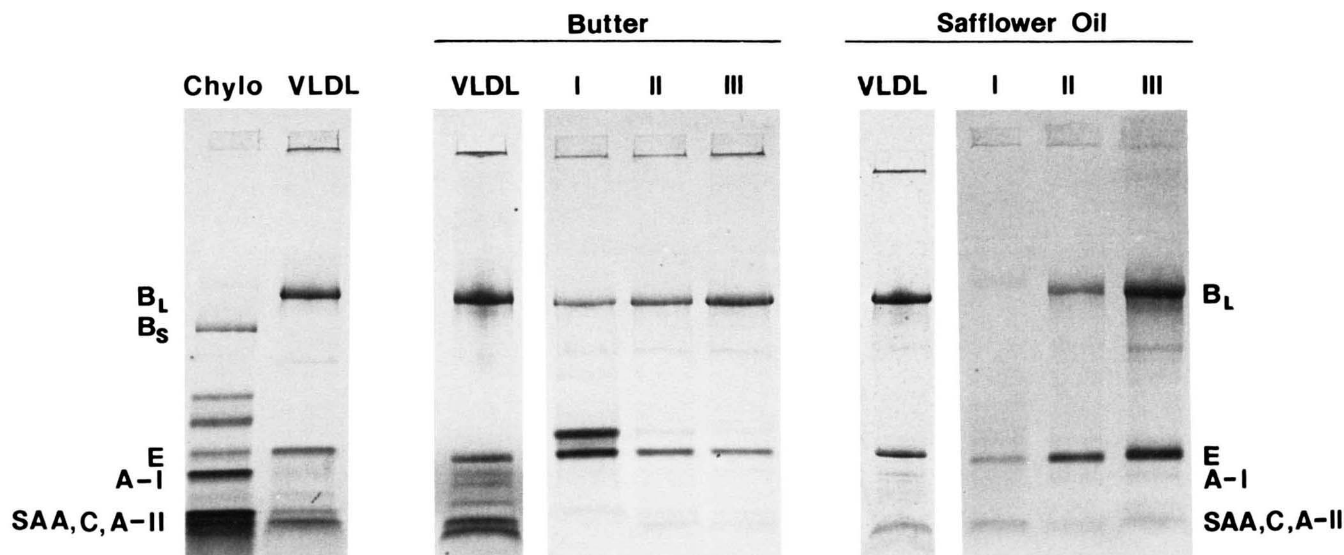


**Fig. 9** SDS-polyacrylamide gradient electrophoresis of liver perfusate lipoproteins. Perfusate lipoproteins were isolated by ultracentrifugation and fractionated by 4% agarose column chromatography; apoproteins were separated by SDS-PAGE using a continuous, linear gradient from 4 to 30% acrylamide. Apoproteins are identified by letters, and lipoprotein lanes are designated as VLDL and column regions I, II, and III from a butter-fed animal and a safflower oil-fed animal. The far left hand two lanes are on the same gel and contain samples of thoracic duct lymph chylomicrons, (containing the small molecular weight form of apoB, designated $B_s$) and of liver perfusate VLDL from a butter-fed monkey (containing the large molecular weight form of apoB, designated $B_L$).



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TABLE 4. Chemical composition of perfusate lipoproteins

| | Diet | n | Pr | PL | FC | TG | CE |
|---|---|---|---|---|---|---|---|
| | | | | | % | | |
| VLDL | Butter | 11 | 10.3 ± 0.3 | 23.1 ± 0.7 | 6.0 ± 0.3 | 50.9 ± 1.7 | 9.8 ± 1.8 |
| | Safflower oil | 8 | 10.9 ± 0.9 | 24.0 ± 1.2 | 6.2 ± 0.5 | 45.1 ± 1.5 | 13.8 ± 1.4 |
| $I_{1.02-1.065}$[a] | Butter | 10 | 14.0 ± 1.3 | 48.6 ± 2.1 | 17.6 ± 1.2 | 12.0 ± 1.7 | 7.9 ± 1.6 |
| | Safflower oil | 6 | 11.3 ± 0.7 | 50.9 ± 2.4 | 19.7 ± 1.7 | 10.8 ± 3.3 | 7.4 ± 1.5 |
| $II_{1.006-1.02}$ | Butter | 11 | 12.9 ± 0.4 | 28.0 ± 1.1 | 8.4 ± 0.4 | 38.5 ± 2.0 | 12.3 ± 1.8 |
| | Safflower oil | 6 | 14.0 ± 1.4 | 28.7 ± 0.9 | 8.8 ± 0.8 | 26.9 ± 2.1 | 21.7 ± 3.1 |
| $II_{1.02-1.03}$ | Butter | 6 | 15.3 ± 0.2 | 36.2 ± 2.9 | 12.8 ± 0.9 | 27.0 ± 2.4 | 8.8 ± 2.0 |
| | Safflower oil | 5 | 15.6 ± 1.5 | 35.2 ± 2.4 | 12.2 ± 1.5 | 16.8 ± 2.0 | 20.1 ± 3.3 |
| $II_{1.03-1.065}$ | Butter | 6 | 17.5 ± 0.7 | 49.4 ± 2.4 | 18.4 ± 0.6 | 9.9 ± 1.9 | 4.5 ± 1.2 |
| | Safflower oil | 5 | 15.7 ± 1.5 | 47.7 ± 3.0 | 17.3 ± 2.0 | 9.3 ± 2.5 | 10.0 ± 2.2 |
| $III_{1.006-1.02}$ | Butter | 5 | 16.5 ± 1.3 | 26.5 ± 1.2 | 8.0 ± 0.6 | 39.6 ± 2.8 | 9.4 ± 2.1 |
| | Safflower oil | 7 | 15.7 ± 1.1 | 27.8 ± 0.7 | 8.2 ± 0.6 | 28.7 ± 1.6 | 19.6 ± 2.7 |
| $III_{1.02-1.03}$ | Butter | 3 | 19.0 ± 1.8 | 29.7 ± 0.6 | 9.1 ± 0.4 | 29.2 ± 2.4 | 12.9 ± 3.5 |
| | Safflower oil | 5 | 18.9 ± 1.6 | 29.3 ± 0.7 | 10.0 ± 0.8 | 21.4 ± 1.9 | 20.6 ± 3.5 |
| $III_{1.03-1.065}$ | Butter | 6 | 20.0 ± 0.8 | 37.4 ± 2.8 | 11.7 ± 0.7 | 22.9 ± 3.0 | 8.0 ± 1.8 |
| | Safflower oil | 5 | 21.6 ± 1.7 | 38.7 ± 2.2 | 14.9 ± 1.4 | 11.8 ± 1.9 | 13.0 ± 3.4 |

[a]Subfractions are designated by elution region by column chromatography on 4% agarose (Roman numerals) followed by a subscript indicating the density range in g/ml of the subfraction.

in livers from butter-fed animals was similar to that of the perfusate VLDL from these livers, being about 45% oleate, 20% linoleate, and 20% palmitate. Likewise, in safflower oil-fed animals, liver cholesteryl esters resembled those of the perfusate VLDL. The cholesteryl esters from both liver and perfusate VLDL from safflower oil-fed animals were about 70% linoleate, 20% oleate, and 3% palmitate. The plasma LDL from butter- and safflower oil-fed animals have cholesteryl ester compositions similar to those of the perfusate VLDL from the two diet groups, respectively (7).

## DISCUSSION

**Fig. 12** is a schematic diagram summarizing the major observations from the present studies and the conclusions the authors have made. The degree of dietary fat saturation influenced the hepatic cholesteryl ester content with

TABLE 5. Rate of accumulation of perfusate lipoproteins

| | Diet | n | Pr | PL | FC | TG | CE | Total |
|---|---|---|---|---|---|---|---|---|
| | | | | | *μg/hr per 100 g* | | | |
| VLDL | Butter | 9 | 712 ± 130 | 1600 ± 295 | 410 ± 79 | 3802 ± 720 | 603 ± 115 | 7127 ± 1270 |
| | Safflower oil | 7 | 680 ± 139 | 1555 ± 376 | 357 ± 70 | 2904 ± 631 | 981 ± 264 | 6477 ± 1435 |
| $I_{1.02-1.065}$[a] | Butter | 8 | 288 ± 63 | 853 ± 131 | 300 ± 53 | 213 ± 45 | 146 ± 59 | 1800 ± 322 |
| | Safflower oil | 7 | 61 ± 17 | 275 ± 69 | 102 ± 27 | 92 ± 29 | 52 ± 24 | 582 ± 138 |
| $II_{1.006-1.02}$ | Butter | 5 | 114 ± 22 | 215 ± 47 | 63 ± 17 | 339 ± 63 | 69 ± 17 | 800 ± 154 |
| | Safflower oil | 5 | 74 ± 8 | 139 ± 19 | 40 ± 5 | 127 ± 13 | 121 ± 35 | 501 ± 72 |
| $II_{1.02-1.03}$ | Butter | 6 | 95 ± 19 | 218 ± 41 | 77 ± 14 | 175 ± 45 | 59 ± 21 | 624 ± 130 |
| | Safflower oil | 5 | 66 ± 12 | 151 ± 29 | 54 ± 13 | 69 ± 11 | 89 ± 26 | 429 ± 81 |
| $II_{1.03-1.065}$ | Butter | 6 | 222 ± 57 | 626 ± 154 | 236 ± 60 | 156 ± 74 | 79 ± 44 | 1319 ± 374 |
| | Safflower oil | 5 | 89 ± 7 | 284 ± 44 | 104 ± 19 | 52 ± 12 | 56 ± 10 | 585 ± 61 |
| $III_{1.006-1.02}$ | Butter | 5 | 126 ± 28 | 199 ± 35 | 62 ± 12 | 315 ± 78 | 70 ± 18 | 772 ± 155 |
| | Safflower oil | 5 | 282 ± 30 | 482 ± 58 | 145 ± 21 | 488 ± 79 | 398 ± 85 | 1796 ± 250 |
| $III_{1.02-1.03}$ | Butter | 3 | 131 ± 12 | 215 ± 45 | 65 ± 12 | 203 ± 21 | 102 ± 47 | 716 ± 135 |
| | Safflower oil | 5 | 337 ± 64 | 519 ± 92 | 181 ± 41 | 374 ± 73 | 454 ± 152 | 1865 ± 393 |
| $III_{1.03-1.065}$ | Butter | 6 | 272 ± 61 | 496 ± 110 | 153 ± 30 | 351 ± 123 | 111 ± 29 | 1383 ± 342 |
| | Safflower oil | 5 | 232 ± 63 | 422 ± 115 | 161 ± 42 | 121 ± 32 | 168 ± 86 | 1104 ± 326 |

[a]Subfractions are designated by elution region by column chromatography on 4% agarose (Roman numerals) followed by a subscript indicating the density range in g/ml of the subfraction.



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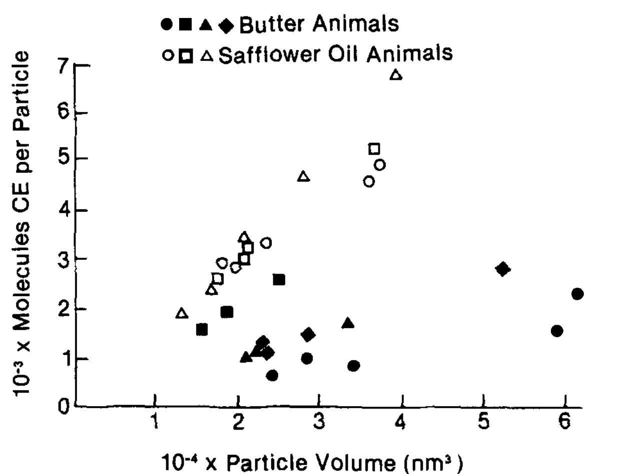


**Fig. 10** Cholesteryl ester content of liver perfusate VLDL. VLDL were isolated from the perfusates by ultracentrifugation and fractionated by 2% agarose column chromatography. The diameters of VLDL within each subfraction were measured by negative stain electron microscopy in order to calculate particle volume. The number of molecules of cholesteryl ester contained per particle was calculated from particle volume measurements and chemical compositions of VLDL subfractions. VLDL subfractions are shown from four animals fed the butter diet (closed symbols) and from three animals fed the safflower oil diet (open symbols).

the livers of polyunsaturated fat-fed animals containing more cholesteryl ester than for those of the saturated fat-fed animals (Fig. 11). This increase is accompanied by an increased secretion of cholesteryl esters by the livers of the polyunsaturated fat group compared to those of the saturated fat group (overall approximately a twofold higher rate as seen in the data of Table 5). The hepatic particles from the polyunsaturated fat group were smaller on the average (Fig. 4, Table 3) but appeared to contain more cholesteryl ester per particle compared to those from the butter group (Fig. 10). However, during conversion of the hepatic lipoproteins into LDL in the circulation, much cholesteryl ester must be removed from the particles of the polyunsaturated group because the plasma LDL are smaller and contain significantly less cholesteryl ester per particle (Table 1). This presumably occurs as a result of cholesteryl ester transfer reactions mediated by the cholesteryl ester exchange protein (CEEP) in the plasma, or it is possibly due to a more rapid clearance from plasma of the cholesteryl ester-enriched lipoproteins secreted by livers of the polyunsaturated fat-fed animals. In the butter group, however, the hepatic lipoproteins may actually require the net gain of cholesteryl ester in order to be converted into plasma LDL. The synthesis of cholesteryl esters by LCAT is presumed to occur at all times in the plasma in both diet groups; preliminary data have indicated the possibility that LCAT may be more active in the saturated fat-fed group (19). The relatively slower removal from plasma of cholesteryl esters, and possibly the higher rate of LCAT activity, would appear to result in the higher number of cholesteryl ester molecules per LDL particle and the greater concentration of LDL particles found in the plasma of the saturated fat-fed animals.

The finding that in both diet groups the LDL molecular weight from the donor animal's plasma was related to the rate of accumulation of cholesterol in the perfusate of the isolated liver (Fig. 2) led us to conclude that the rate of hepatic cholesterol secretion may be an important determinant of plasma LDL molecular weight, regardless of the degree of dietary fat saturation. However, for a given rate of hepatic cholesterol secretion, the molecular weight of the plasma LDL in the donor animal was predicted to be smaller in safflower oil-fed animals than in butter-fed animals (Fig. 2). Thus, dietary polyunsaturated fat did not lower plasma LDL molecular weight by decreasing hepatic cholesterol secretion. The finding that hepatic lipoproteins from safflower oil-fed animals were enriched in cholesteryl ester compared to particles from butter-fed animals emphasizes this point. Plasma LDL particles contain about 2000–2500 cholesteryl ester molecules depending upon the individual animal and the degree of saturation of the fat in the animal's diet (unpublished observations), whereas the hepatic VLDL particles from safflower oil-fed animals contained 1900–6800 cholesteryl ester molecules, averaging about 3000; hepatic

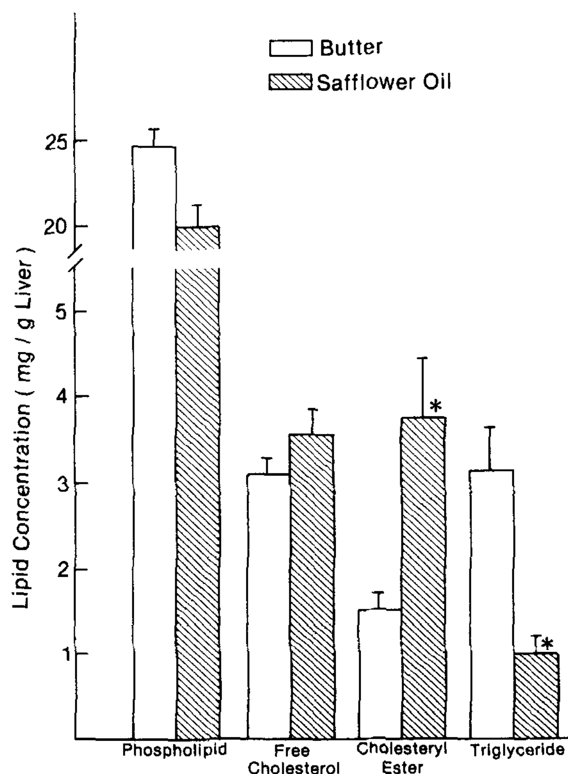


**Fig. 11** Liver lipid concentration. Liver lipids were measured on 12 butter-fed animals (open bars) and 8 safflower oil-fed animals. *Significantly different from butter group, $P < 0.01$.



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TABLE 6. Effects of degree of dietary fat saturation on hepatic and perfusate VLDL cholesteryl ester composition

| Diet | | n | Cholesteryl Ester Composition 16:0[a] | 18:0 | 18:1 | 18:2 | 20:4 | Others |
|---|---|---|---|---|---|---|---|---|
| | | | % | | | | | |
| Butter | Liver | 9 | 15.6 ± 1.8 | 6.1 ± 1.5 | 46.7 ± 1.5 | 25.4 ± 2.2 | 5.3 ± 0.9 | 1.2 ± 0.8 |
| | Liver VLDL | 6 | 23.6 ± 1.2 | 9.3 ± 2.3 | 42.9 ± 1.7 | 21.4 ± 2.6 | 0.9 ± 0.5 | 1.9 ± 0.7 |
| Safflower oil | Liver | 11 | 2.0 ± 0.8 | 2.2 ± 0.8 | 22.1 ± 1.0 | 63.8 ± 1.8 | 5.1 ± 0.4 | 4.8 ± 2.0 |
| | Liver VLDL | 4 | 4.4 ± 1.7 | 1.1 ± 0.7 | 20.0 ± 3.1 | 73.8 ± 3.8 | 0.6 ± 0.4 | 0 |

[a]Fatty acids are designated by chain length: number of double bonds.

VLDL particles from butter-fed animals contained only about 600–3000 cholesteryl ester molecules, averaging about 1300. Therefore, for plasma LDL to be produced from hepatic lipoproteins in vivo, extensive intravascular modification of the newly secreted particles must occur; and in the case of hepatic VLDL from safflower oil-fed animals, this includes the net removal of large amounts of cholesteryl ester.

The cholesteryl ester enrichment of hepatic lipoproteins from safflower oil-fed animals was likely due to increased hepatic secretion of cholesteryl ester and not due to the action of LCAT. Significant LCAT activity was not detectable in the perfused liver system as evidenced by direct assay using the method of Matz and Jonas (20) (data not shown) and by perfusate lipoprotein structure. Liver perfusates from both diet groups contained an abundance of cholesteryl ester-poor, discoidal HDL particles. These lipoproteins were similar in structure and composition to the so-called nascent HDL found in perfusates of rat livers in which LCAT is inhibited (21) and also were similar to the plasma HDL from patients with familial LCAT deficiency (22, 23). The large, phospholipid- and free cholesterol-rich lipoproteins isolated within the LDL density range from liver perfusates also are similar in composition and structure to the plasma LDL from patients with LCAT deficiency (24).

The composition of cholesteryl esters in perfusate lipoproteins of butter-fed animals is similar to that of the liver cholesteryl esters, being rich in saturated and monounsaturated esters, unlike the linoleate-rich cholesteryl esters typically synthesized in plasma by LCAT (25). Both the liver and perfusate lipoprotein cholesteryl esters from safflower oil-fed animals were rich in linoleate, reflecting the composition of this dietary fat. In this case, the relative importance of LCAT is unclear, however the presence of similar amounts of discoidal HDL in perfusates from safflower oil-fed and butter-fed animals suggests that an LCAT origin of the perfusate cholesteryl esters in either diet group is unlikely. Immunoassay for LCAT (kindly performed in the laboratory of Dr. John Albers) indicated that the concentrations in liver perfusates are less than 1% of that in monkey plasma. Based on data from de Parscau and Fielding (26) on the rate of secretion of LCAT activity by perfused rabbit livers, we calculate that LCAT could account for only about 3% of the cholesteryl ester mass in the perfusate lipoproteins in the present studies. The data on the liver perfusate cholesteryl ester and lipoprotein compositions are consistent with a state of LCAT deficiency during perfusion. We conclude that the majority of the lipoprotein cholesteryl esters of the perfusate were secreted directly by the perfused liver.

The stimulus for increased hepatic secretion of cholesteryl ester in animals fed polyunsaturated fat is unclear, but increased hepatic cholesteryl ester content may be one

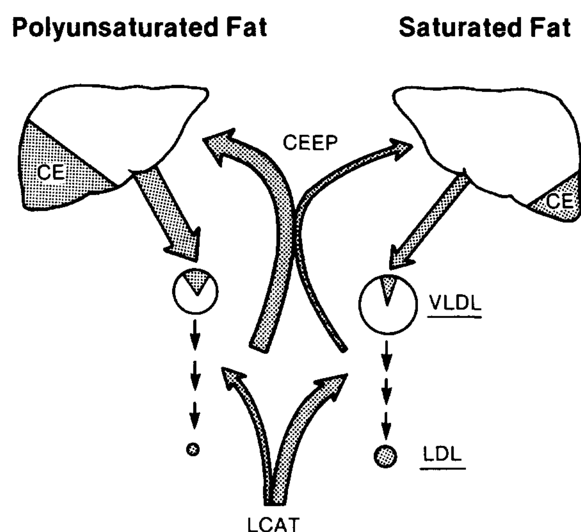


**Fig. 12** Schematic summary integrating the findings of the present study into a metabolic pathway that could account for both in vitro and in vivo observations. The shaded areas of the diagram depict cholesteryl esters. CEEP is the abbreviation for cholesteryl ester exchange protein(s) and represents the exchange and/or net transfer of cholesteryl esters among lipoproteins that occur in plasma. LCAT is the abbreviation for the enzyme lecithin:cholesterol acyltransferase that is acknowledged to be a major source of plasma lipoprotein cholesteryl esters along with the liver in these cholesterol-fed African green monkeys. The size of the lipoproteins and the arrows are different for each diet group where diet effects are believed to occur.



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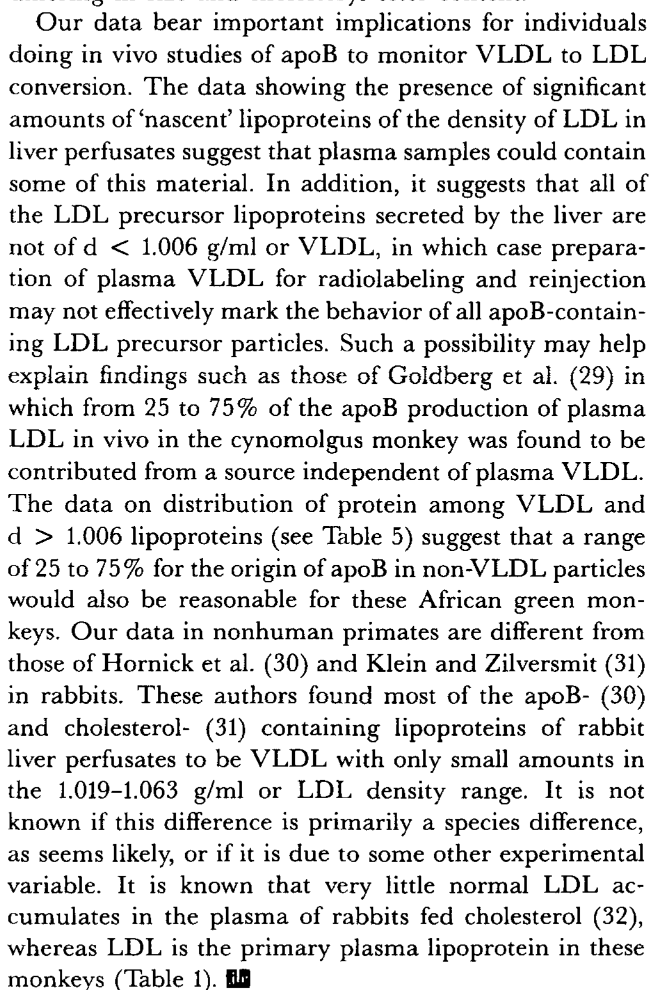
factor. Secretion rates of cholesteryl esters (and triglycerides) may also depend upon the physical state of the individual lipids. The more polyunsaturated cholesteryl esters may remain in a fluid state while more saturated cholesteryl esters, with phase transition temperatures above body temperature, may be in a crystalline state in the hepatocyte and therefore less available for secretion. Glick et al. (27) have provided evidence that the composition and physical state of rat hepatoma cell lipids are important determinants of the clearance of cholesteryl esters from these cells in culture. In our studies the triglyceride content was lower in the livers from the safflower oil-fed monkeys compared to butter-fed monkeys. The effects of different ratios of triglyceride relative to cholesteryl ester and of different degrees of the saturation of triglyceride upon the physical state of stored hepatic lipids may also be of importance in influencing lipoprotein secretion. However, even though the hepatic content of cholesteryl ester was much greater than that of triglyceride in the safflower oil-fed group, the hepatic lipoproteins secreted were generally triglyceride-rich, not cholesteryl ester-rich, indicating the preference of the liver to secrete triglyceride.

As seen in these and in previous studies (6), perfused monkey livers produce a large spectrum of particle types differing not only in the relative amounts of triglyceride and cholesteryl ester but also in the amounts of the surface lipids. Compositionally, it appears as if these particles could represent a continuum of particles of similar metabolic origin but of different sizes and different surface/core ratios, i.e., the d > 1.006 g/ml lipoproteins contain proportionally more surface than the d < 1.006 g/ml lipoproteins. It is possible that the surface lipid-rich perfusate particles are products of postsecretory modification during recirculation perfusion of more nascent hepatic lipoproteins. If such were the case, the relative amounts and chemical composition of the various subfractions secreted by the liver may differ considerably from that isolated after 4 hr of recirculation. Additional studies, such as those utilizing a nonrecirculating perfusion system, are indicated in order to resolve these issues.

Another point should be made regarding the potential source of some of the excess surface lipid seen in the perfusate lipoproteins. Red blood cells were present in our perfusions and it seems possible that some of the excess surface phospholipid and free cholesterol could have originated in these cells. In a sense, apoproteins are detergents and could capture some of the red blood cell lipids that then become associated with the lipoproteins. In the absence of a significant level of LCAT activity, surface remodeling may not have occurred normally, resulting in some of the surface-enriched structures that were seen. Further experiments will be required to determine the extent to which such phenomena contribute to the outcome.

Because the apoB of all of the perfusate lipoproteins was only the large molecular weight form, these newly secreted lipoproteins are potential precursors to plasma LDL. The data of the present report and of our earlier publication (6) show that the densities of these precursors lipoproteins include not only d < 1.006 g/ml (VLDL) but also the 1.006–1.019 g/ml range (IDL) and the 1.019–1.063 g/ml range (LDL). Although particles in each of these conventional density ranges have been identified, their size and percentage compositions show that extensive intravascular modification will be required before they become particles typical of plasma LDL. This variety of apoB-containing, newly secreted hepatic lipoproteins may explain in part the polydispersity of the plasma LDL seen in African green monkeys (1, 4) and in several other species of nonhuman primates (1) and in man (28). Dietary polyunsaturated fat could reduce the average molecular weight of plasma LDL by increasing the number of low molecular weight LDL particles relative to the number of high molecular weight LDL particles. In this regard, one might speculate that hepatic lipoproteins differing in size and cholesteryl ester content may be transformed in the circulation into various plasma LDL differing in size and cholesteryl ester content.



Our data bear important implications for individuals doing in vivo studies of apoB to monitor VLDL to LDL conversion. The data showing the presence of significant amounts of 'nascent' lipoproteins of the density of LDL in liver perfusates suggest that plasma samples could contain some of this material. In addition, it suggests that all of the LDL precursor lipoproteins secreted by the liver are not of d < 1.006 g/ml or VLDL, in which case preparation of plasma VLDL for radiolabeling and reinjection may not effectively mark the behavior of all apoB-containing LDL precursor particles. Such a possibility may help explain findings such as those of Goldberg et al. (29) in which from 25 to 75% of the apoB production of plasma LDL in vivo in the cynomolgus monkey was found to be contributed from a source independent of plasma VLDL. The data on distribution of protein among VLDL and d > 1.006 lipoproteins (see Table 5) suggest that a range of 25 to 75% for the origin of apoB in non-VLDL particles would also be reasonable for these African green monkeys. Our data in nonhuman primates are different from those of Hornick et al. (30) and Klein and Zilversmit (31) in rabbits. These authors found most of the apoB- (30) and cholesterol- (31) containing lipoproteins of rabbit liver perfusates to be VLDL with only small amounts in the 1.019–1.063 g/ml or LDL density range. It is not known if this difference is primarily a species difference, as seems likely, or if it is due to some other experimental variable. It is known that very little normal LDL accumulates in the plasma of rabbits fed cholesterol (32), whereas LDL is the primary plasma lipoprotein in these monkeys (Table 1).



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## REFERENCES

1. Rudel, L. L., L. L. Pitts, and C. A. Nelson. 1977. Characterization of plasma low density lipoproteins of nonhuman primates fed dietary cholesterol. *J. Lipid Res.* **18:** 211-222.
2. Rudel, L. L., C. W. Leathers, M. G. Bond, and B. C. Bullock. 1981. Dietary ethanol-induced modifications in hyperlipoproteinemia and atherosclerosis in nonhuman primates (*Macaca nemestrina*). *Arteriosclerosis.* **1:** 144-155.
3. Rudel, L. L. 1980. Plasma lipoproteins in atherogenesis in nonhuman primates. *In* Use of Nonhuman Primates in Cardiovascular Research. S. S. Kalter, editor. University of Texas Press, Austin. 37-57.
4. Rudel, L. L., and B. C. Bullock. 1981. Low density lipoprotein-atherosclerosis relationships in African green monkeys. *Federation Proc.* **40:** 345 (Abstract).
5. Rudel, L. L., J. S. Parks, and R. M. Carroll. 1983. Effects of polyunsaturated versus saturated dietary fat on nonhuman primate HDL. *In* Dietary Fats and Health. E. G. Perkins, and W. J. Visek, editors. American Oil Chemists' Society, Champaign, IL. 649-666.
6. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. Effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221-236.
7. Rudel, L. L., and D. G. Greene. 1978. Compositional studies of low density lipoproteins (LDL). *Federation Proc.* **37:** 1322 (Abstract).
8. Rudel, L. L., J. A. Reynolds, and B. C. Bullock. 1981. Nutritional effects on blood lipid and HDL cholesterol concentrations in two subspecies of African green monkeys. (*Cercopithecus aethiops*). *J. Lipid Res.* **22:** 278-286.
9. Rudel, L. L., J. A. Lee, M. D. Morris, and J. M. Felts. 1974. Characterization of plasma lipoproteins separated and purified by agarose-column chromatography. *Biochem. J.* **139:** 89-95.
10. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497-509.
11. Rudel, L. L., and M. D. Morris. 1973. Determination of cholesterol using *o*-phthalaldehyde. *J. Lipid Res.* **14:** 364-366.
12. Fiske, C. A., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 375-400.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.
14. Sardesai, V. M., and J. A. Manning. 1968. The determination of triglycerides in plasma and tissues. *Clin. Chem.* **14:** 156-161.
15. Carroll, R. M., and L. L. Rudel. 1981. Evaluation of a high-performance liquid chromatography method for isolation and quantitation of cholesterol and cholesteryl esters. *J. Lipid Res.* **22:** 359-363.
16. Weeke, B. 1972. Rocket immunoelectrophoresis. *Scand. J. Immunol.* **2 (Suppl. 1):** 15-46.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature.* **227:** 680-685.
18. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757-768.
19. Carroll, R. M., and L. L. Rudel. 1981. Dietary fat and cholesterol effects on lipoprotein cholesterol ester formation via lecithin:cholesterol acyltransferase (LCAT) in vervet monkeys. *Federation Proc.* **40:** 1695 (Abstract).
20. Matz, C. E., and A. Jonas. 1982. Reaction of human lecithin:cholesterol acyltransferase with synthetic micellar complexes of apolipoprotein A-I, phosphatidylcholine, and cholesterol. *J. Biol. Chem.* **257:** 4541-4546.
21. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667-680.
22. Torsvik, H., M. H. Solaas, and E. Gjone. 1970. Serum lipoproteins in plasma lecithin:cholesterol acyltransferase deficiency, studied by electron microscopy. *Clin. Genet.* **1:** 139-150.
23. Forte, T., K. R. Norum, J. A. Glomset, and A. V. Nichols. 1971. Plama lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy. *J. Clin. Invest.* **50:** 1141-1148.
24. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase: perspective from pathology. *Adv. Lipid Res.* **11:** 1-65.
25. Glomset, J. A. 1962. The mechanism of the plasma cholesterol esterification reaction of plasma fatty acid transferase. *Biochim. Biophys. Acta.* **65:** 128-135.
26. de Parscau, L., and P. E. Fielding. 1983. Lecithin:cholesterol acyltransferase and cholesterol ester transfer activity from the isolated perfused rabbit liver. *Circulation* **68 (Suppl. III):** 927 (Abstract).
27. Glick, J. M., S. J. Adelman, M. C. Phillips, and G. H. Rothblat. 1983. Cellular cholesteryl ester clearance. Relationship to the physical state of cholesteryl ester inclusions. *J. Biol. Chem.* **258:** 13425-13430.
28. Krauss, R. M., and D. J. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23:** 97-104.
29. Goldberg, I. J., N-A. Le, H. N. Ginsberg, J. R. Paterniti, Jr., and W. V. Brown. 1983. Metabolism of apoprotein B in cynomolgus monkey: evidence for independent production of low density lipoprotein apoprotein B. *Am. J. Physiol.* **244:** E196-E201.
30. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe heritable hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* **80:** 6096-6100.
31. Klein, R. L., and D. B. Zilversmit. 1984. Hepatic very low density lipoprotein (VLDL) subfractions are probably precursors to plasma VLDL subfractions in the cholesterol-fed rabbit. *Federation Proc.* **43:** 480 (Abstract).
32. Shore, V. G., B. Shore, and R. G. Hart. 1974. Changes in apolipoproteins and properties of rabbit very low density lipoproteins on induction of cholesterolemia. *Biochemistry.* **13:** 1579-1584.



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