

Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal

Jeffrey S. Cohn, Judith R. McNamara, Susan D. Cohn, Jose M. Ordovas, and Ernst J. Schaefer

Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington St., Boston, MA 02111

Abstract Twenty two subjects (9 males, 13 females) were fed a fat-rich meal (1 g of fat/kg body weight). Triglyceride-rich lipoproteins (TRL) were isolated by ultracentrifugation ($d < 1.006$ g/ml) from blood drawn 0, 3, 6, 9, and 12 hr after the meal. Plasma triglyceride increased then decreased postprandially, while plasma apoA-I and apoB concentrations decreased. TRL triglyceride, TRL total protein, and TRL apoB concentrations all increased then decreased after the fat-rich meal. Postprandial rise in plasma triglyceride was significantly correlated with fasting plasma triglyceride levels ($r = 0.66$, $P < 0.001$); postprandial rise in TRL triglyceride was significantly correlated with fasting TRL triglyceride levels ($r = 0.58$, $P < 0.01$); postprandial rise in TRL apoB was not, however, significantly correlated with fasting TRL apoB levels ($r = 0.37$, N.S.). TRL apolipoproteins were separated by polyacrylamide gradient (4–22.5%) gel electrophoresis and protein bands were scanned in two dimensions with a laser densitometer. Relative postprandial changes in the concentration of the TRL apolipoproteins were determined. TRL apoB-100, apoB-48, apoE, and apoC increased then decreased postprandially. The increase in TRL apoB-100 after the fat-rich meal was confirmed in 8 subjects by direct measurement of apoB-100 with a monoclonal antibody ELISA assay. ApoA-I concentration in TRL was unchanged. Albumin in the TRL fraction was significantly increased 12 hr after the meal. Subjects with a greater magnitude of postprandial triglyceridemia had a greater increase in TRL triglyceride and TRL apoB, but their TRL apoB-100/apoB-48 ratios were not different from subjects with less pronounced triglyceridemia. ■ Assuming that plasma TRL containing apoB-100 are predominantly derived from the liver, our data suggest that triglyceride-rich lipoproteins from both the liver and intestine make a significant contribution to postprandial triglyceridemia. — Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *J. Lipid Res.* 1988. 29: 925–936.

Supplementary key words postprandial triglyceridemia • chylomicrons • apolipoprotein B-100 • apolipoprotein B-48

In the fasting state, triglyceride is secreted by the liver in very low density lipoproteins (VLDL). The majority of

these lipoproteins are converted to low density lipoproteins (LDL), which are cleared from the circulation by specific LDL (apoB) receptors in the liver and peripheral tissues (1). Following the ingestion of a fat-rich meal, there is a postprandial increase in plasma triglyceride due to the secretion of triglyceride-rich chylomicrons from the intestine (2). Chylomicron triglycerides are rapidly hydrolyzed in peripheral tissues by lipoprotein lipase and are subsequently cleared from the plasma by specific chylomicron remnant (apoE) receptors in the liver (3).

The triglyceride-rich lipoproteins (TRL) in plasma contain two forms of apoB (4, 5): a high molecular weight protein, apoB-100, and a lower molecular weight protein, apoB-48. The liver in humans synthesizes only apoB-100 (6), whereas the intestine synthesizes apoB-48. Recent *in vitro* evidence suggests that the intestine can also synthesize apoB-100 (7, 8); however there is strong evidence that *in vivo* the adult intestine synthesizes only apoB-48 (9, 10). Although this issue remains controversial, it would appear that apoB-100 in plasma is predominantly of hepatic origin.

In a previous report (11), we documented the lipoprotein lipid changes that occurred in a group of human subjects fed a fat-rich meal. In the present study, we have investigated the apolipoprotein composition of the TRL from these subjects. We have measured relative TRL apoB-100 and apoB-48 concentrations in order to establish the relative contribution of these two species of TRL to postprandial triglyceridemia.

Abbreviations: TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein; EDTA, ethylenediamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulphate.

METHODS

Human subjects

Twenty two healthy subjects (9 males, 13 females) were studied. They ranged in age from 22 to 79 years. Subjects were selected who had no history of ulcer disease or bleeding tendency. They were not taking medications known to affect plasma lipids. Their fasting plasma triglyceride, plasma cholesterol, and high density lipoprotein (HDL) cholesterol concentrations (Table 1) were within normal ranges for their age groups. All studies were carried out in the Metabolic Unit of the USDA Human Nutrition Research Center. The experimental protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University.

Study design

The protocol for the fat-feeding experiments has been described previously (11). After a 14-hr overnight fast, subjects were given a fat meal, containing 1.0 g of fat/kg body weight and 7.0 mg of cholesterol/kg (given as egg yolk powder). The fat was given as soybean oil to 12 subjects and as soybean oil plus cream (1:1, w/w) to the remaining 10 subjects. Since no differences were observed between subjects fed the two fat mixtures, data were analyzed together for all 22 subjects. The amount of fat given was designed to be $\frac{1}{2}$ - $\frac{2}{3}$ of that which an average American ingests per day. The meal was prepared as a formula milkshake with added flavoring. Polycose and egg white protein were added to the formula so that the meal contained 33.3% fat, 33.3% protein, and 33.3% carbohydrate (by weight) or 53% fat, 23.5% protein, 23.5% carbohydrate (by energy). Blood samples (20 ml) were obtained via a small forearm indwelling catheter prior to the test meal and at 3, 6, 9, and 12 hr after the meal. Water, but no food, was allowed during the study period.

Preparation of lipoproteins

Blood was collected in tubes containing EDTA to give a final concentration of 0.1% EDTA. Plasma was separated from red blood cells by centrifugation (2,500 rpm) for 20 min at 4°C. The triglyceride-rich lipoproteins

(TRL), consisting of chylomicrons and very low density lipoproteins (VLDL), were isolated from 5 ml of plasma by a single ultracentrifugal spin (39,000 rpm, 18 hr, 4°C) at density 1.006 g/ml in a Beckman 50.3Ti rotor. TRL fractions were not recentrifuged. Fraction volumes were adjusted to 3 ml with normal saline and they were assayed for protein by the method of Lowry et al. (12). Turbidity was cleared with chloroform. Samples were aliquoted, frozen, and stored at -70°C until time of analysis.

Lipoproteins can be stripped of a small proportion of their apolipoproteins during ultracentrifugal isolation. This represents an inherent shortcoming of this methodology. We nevertheless chose this method of separation because we were predominantly interested in the B apolipoproteins, which are not readily lost from TRL. Furthermore, this method of separation allowed us to efficiently isolate multiple TRL samples with good quantitative reproducibility, something that cannot be readily achieved with other methods.

Lipid analysis

Plasma and lipoprotein fractions were assayed for total cholesterol and triglyceride with an Abbott Diagnostics ABA-200 bichromatic analyzer using enzymatic reagents, as previously described (13). Assays were standardized through participation in the Centers for Disease Control-National Heart, Lung, and Blood Institute Standardization program. HDL cholesterol was quantitated by analyzing the supernate obtained following precipitation of a plasma aliquot with dextran sulfate-Mg²⁺, as described by Warnick, Benderson, and Albers (14).

Apolipoprotein quantitation

Plasma and TRL apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB = apoB-100 + apoB-48) were measured by a noncompetitive enzyme-linked immunosorbent assay (ELISA) (15). Polystyrene microtiter plates (Nunc Immunoplate 1, Nunc, Denmark) were coated with affinity-purified polyclonal antibodies to human apoA-I or apoB. Duplicate plasma samples were diluted 1 in 3,000 for the apoB assay or 1 in 60,000 for the apoA-I assay with phosphate-buffered saline, and were then added to desig-

TABLE 1. Characteristics of human subjects

Subjects	Age	Body Mass Index	Plasma Triglyceride Concentration	Plasma Cholesterol Concentration	HDL Cholesterol Concentration
	<i>yr</i>	<i>kg/m²</i>		<i>mg/dl</i>	
Males (n = 9)	49 ± 7	24.9 ± 1.6	121 ± 21	179 ± 10	53 ± 3
Females (n = 13)	52 ± 5	23.0 ± 0.9	89 ± 9	192 ± 11	56 ± 3
All subjects (n = 22)	51 ± 4	23.8 ± 0.8	102 ± 10	187 ± 8	55 ± 3

Values are means ± SEM.

nated wells in the microtiter plates along with standards and controls. After incubation overnight, immunopurified apoB or apoA-I antibody conjugated to alkaline phosphatase was added. Color development was achieved with the addition of a substrate (0.1% *p*-nitrophenyl phosphate in 0.1 M glycine buffer). After 20 min, the plates were read at 410 nm on a microtiter plate reader (Dynatech MR600, Dynatech Inc., Vienna, VA), interfaced with an IBM XT microcomputer, programmed with Immunosoft (Dynatech). ApoB-100 was measured by ELISA assay using the same procedure, however, a monoclonal antibody B-3 (RIA Inc., Scarborough, Canada), which did not react with apoB-48, was used to coat the plates.

Separation and quantitation of apolipoproteins by gradient gel electrophoresis

The apolipoproteins in the TRL fraction were separated by polyacrylamide gel electrophoresis in the presence of SDS on 1.5-mm thick vertical slab gels. Electrophoresis was carried out using the buffer system described by Laemmli (16). Gels contained a 4–22.5% acrylamide gradient, 0.1% SDS, and 0.375 M Tris. The acrylamide gradient was poured with the use of a Hoefer SG-50 two-chamber, gravity-flow gradient maker (Hoefer, San Francisco, CA). A stacking gel (3% acrylamide, 0.1% SDS, 0.125 M Tris, pH 6.8) was added with a 10-slot well-forming comb in position.

Nondelipidated lipoprotein samples (25 μ g of protein) were reduced in SDS sample buffer (3% SDS, 3% mercaptoethanol, 0.001% bromophenol blue, 0.05 M Tris, 10% glycerol, pH 6.8) for 10 min at 80°C. Gels were run overnight at 50 V for approximately 16 hr until the dye front was 0.5 cm from the bottom of the plate. Gels were stained for 1 hr with 0.1% Coomassie Brilliant Blue in methanol–acetic acid–water 3:1:6. Destaining was achieved by several changes of the same solvent. The two molecular weight forms of apoB were clearly separated in this gel system. Apolipoproteins were identified by comparing the distance they migrated into the gels with known molecular weight standards (High Molecular Weight Standard Mixture, Sigma Chemical Co.). The apoB-100 band of TRL comigrated with the only stainable band in a narrow-cut LDL fraction ($1.040 < d < 1.050$ g/ml). The apoB-48 band of TRL comigrated with the major high molecular weight band of lymph chylomicrons. The identity of the apoB bands was confirmed by Western blotting analysis using a polyclonal antibody that was known to react with both apoB-100 and apoB-48.

Gradient gels were scanned with an LKB Ultrosan XL laser densitometer linked to an AT&T 6300 personal computer. Apolipoprotein bands were scanned in two dimensions and staining intensity was quantitated with the use of an LKB 2400 Gelscan XL software package. Staining was measured in arbitrary units (a.u.) to two decimal

places. Each lane was routinely scanned and integrated three times. Since it is well accepted that the chromogenicity of individual apolipoproteins is different (17, 18), no attempt was made to quantitate the apolipoproteins in absolute terms. A qualitative, rather than quantitative method was thus devised to measure postprandial changes in the TRL apolipoproteins. The concentration of each TRL apolipoprotein at time points after the fat-rich meal was calculated as a percentage of its zero-hr (fasting) concentration, according to the following formula:

$$\frac{X(\text{a.u.}) \times C_t (\text{mg/dl})}{Y(\text{a.u.}) \times C_o (\text{mg/dl})} \times 100$$

where X = staining intensity of apolipoprotein band at time point after the fat meal; Y = staining intensity of apolipoprotein band at zero time; a.u. = arbitrary units; C_t = TRL total protein concentration in plasma at time point after the fat meal; and C_o = TRL total protein concentration in plasma at zero time.

Comparative analysis by this method depends upon linearity of staining of apolipoprotein bands. Linearity was tested by loading increasing amounts of TRL protein onto a gradient gel. After electrophoresis and staining, bands were scanned in two dimensions with the laser densitometer. Results for apoB-100, apoB-48, apoE, and apoC are shown in Fig. 1. Linearity of staining occurred up to ca. 40 μ g of TRL protein loaded. Similar linearity was observed for the other TRL apolipoprotein bands (data not shown). Subsequently, 25 μ g of sample protein was chosen as an optimal amount to load onto gels, so that apolipoprotein bands fell in the linear range of staining intensity.

Quantitation of postprandial triglyceridemia

Postprandial triglyceridemia was measured by planimetry from the graph of plasma triglyceride concentration (measured hourly) plotted against time after the fat meal. Total postprandial triglyceridemia (measured in mg · hr/dl) was calculated as the area between the curve and baseline (zero concentration). Postprandial change in TRL triglyceride and TRL apoB (measured trihourly) was also quantitated from graphs of TRL component concentrations plotted against time after the fat meal. Change was quantitated as the area between the curves and a line drawn through the zero hour (fasting) concentration.

Statistics

Data were tabulated and stored in a VAX-780 computer (Digital Equipment Co., Maynard, MA), using the scientific software package RS/1 (BBN Research Systems). Statistical analysis was carried out with Statistical Analysis System software (SAS Institute Inc., Cary, NC). Paired and unpaired *t*-tests were used to compare the significance

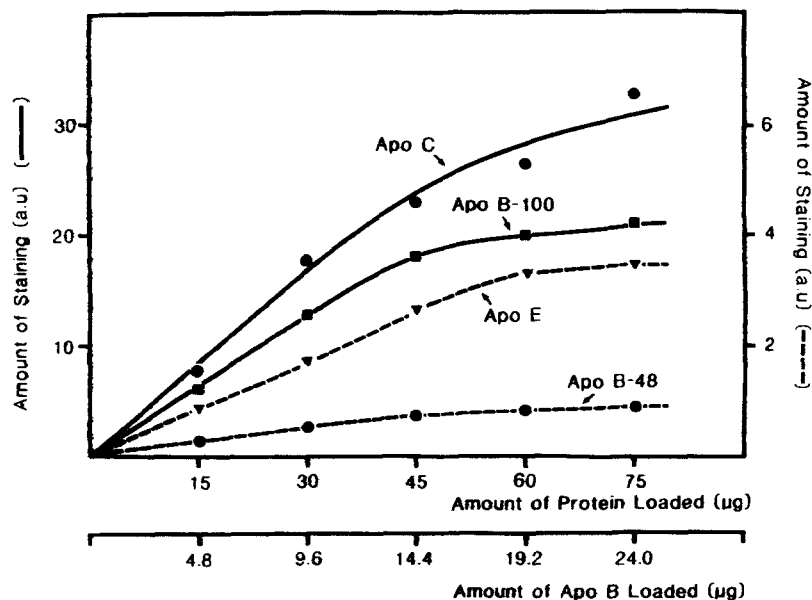


Fig. 1. Chromogenicity of the TRL apolipoproteins. Increasing amounts of TRL protein were loaded onto gradient gels. Gels were electrophoresed, stained, and then scanned with a two-dimensional laser densitometer. Intensity of staining for each protein band was measured in arbitrary units (a.u.), as the average of three measurements made on the same sample. Each sample was run in duplicate. Data for the remaining TRL apolipoproteins (apoH, apoA-IV, and apoA-I) are not shown. Linearity of staining was observed for all TRL apolipoproteins when less than 45 µg of total TRL protein was loaded.

of mean differences. Pearson correlation coefficients (r) were computed to identify significant correlations between lipid parameters.

RESULTS

Plasma triglyceride concentration increased in all subjects fed the fat-rich meal. As shown in Table 2, the mean plasma triglyceride concentration was significantly increased 3, 6, and 9 hr after ingestion of the fatty meal. The mean plasma cholesterol concentration did not change postprandially, although a small increase in plasma cholesterol was observed in some subjects, while

a small decrease was observed in others. Plasma apoA-I and apoB concentrations decreased slightly after the meal (Table 2).

Triglyceride-rich lipoproteins (TRL) were separated from plasma by ultracentrifugation and their composition was analyzed. TRL triglyceride, TRL total protein, and TRL apoB measurements are shown in Table 3. The majority of plasma triglyceride was isolated in the TRL fraction: 67% in the fasting state, 84%, 3 hr postprandially. The plasma concentration of all three TRL components increased postprandially. They were significantly elevated 3 and 6 hr after the fat-rich meal. TRL triglyceride, protein, and apoB did not increase proportionately, however, and as depicted by the TRL triglyceride/protein ratio

TABLE 2. Plasma triglyceride, cholesterol, apolipoprotein A-I and apolipoprotein B concentrations following the ingestion of the fat-rich meal

	Plasma Concentration at Times after Ingestion of Fat-Rich Meal				
	0 Hour ^a	3 Hour	6 Hour	9 Hour	12 Hour
	<i>mg/dl</i>				
Triglyceride	102 ± 10	230 ± 22***	213 ± 25***	152 ± 20**	96 ± 15
Cholesterol	187 ± 8	184 ± 8	185 ± 8	185 ± 7	183 ± 7
Apolipoprotein A-I	168 ± 7	160 ± 7***	160 ± 7**	162 ± 6*	162 ± 6*
Apolipoprotein B	80 ± 4	78 ± 4	76 ± 4*	75 ± 4**	75 ± 4*

Values are means ± SEM for 22 subjects. Results are significantly different from zero-time by paired *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^aZero-hr concentrations were measured after a 14-hr overnight fast, before the ingestion of the fat-rich meal.

TABLE 3. Postprandial changes in the triglyceride-rich lipoprotein (TRL) fraction after the fat-rich meal

	Time after Ingestion of Fat-Rich Meal				
	0 Hour ^a	3 Hour	6 Hour	9 Hour	12 Hour
TRL triglyceride (mg/dl)	73 ± 10	199 ± 21***	179 ± 23***	105 ± 17*	66 ± 15
TRL protein (mg/dl)	15.8 ± 1.4	22.1 ± 1.9***	22.8 ± 2.1***	17.2 ± 1.8	14.8 ± 2.2
TRL apoB (mg/dl) ^b	4.5 ± 0.8	6.2 ± 0.9***	6.5 ± 0.9***	4.9 ± 1.1	3.1 ± 1.0*
TRL triglyceride/protein ratio	4.3 ± 0.3	8.9 ± 0.6***	7.3 ± 0.5***	5.8 ± 0.5**	4.1 ± 0.3
TRL apoB/protein ratio (%)	25.4 ± 2.6	26.5 ± 2.2	26.2 ± 2.3	20.8 ± 2.7	15.5 ± 2.5***
TRL triglyceride/plasma triglyceride ratio (%)	67.0 ± 2.8	84.1 ± 1.6***	79.8 ± 2.5***	68.7 ± 2.8	59.2 ± 3.5**

Values are means ± SEM for 22 subjects. Significantly different from zero time by paired *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^aZero-hr concentrations were measured after a 14-hr overnight fast, before the ingestion of the fat-rich meal.

^bTRL apoB was measured by ELISA assay using a polyclonal antibody that recognized both apoB-100 and apoB-48.

(Table 3), lipoproteins in this fraction were enriched with triglyceride 3, 6, and 9 hr postprandially. The TRL apoB/protein ratio remained relatively constant, but was significantly lower at 12 hr. ApoB in the $d > 1.006$ g/ml fraction of plasma (predominantly LDL apoB) was calculated as the difference between total plasma apoB and TRL apoB. Mean fasting and postprandial apoB concentrations in the $d > 1.006$ fraction were: 0 hr: 75 ± 4 mg/dl; 3 hr: 72 ± 4 mg/dl; 6 hr: 69 ± 4 mg/dl; 9 hr: 70 ± 4 mg/dl; 12 hr: 72 ± 4 mg/dl. ApoB concentration in the $d > 1.006$ g/ml fraction was significantly ($P < 0.05$) lower than the fasting concentration at 3, 6, and 9 hr after the fat-rich meal.

Postprandial change in TRL triglyceride and TRL apoB was quantitated by planimetry from plasma response curves (see Methods). The magnitude of postprandial change in these TRL components varied considerably between subjects. Some of this variability was due to the different ages and sex of the subjects studied. Males ($n = 9$) tended to have a greater postprandial increase in TRL triglyceride (1074 ± 182 vs. 709 ± 139 mg·hr/dl) (mean ± SEM) and TRL apoB (17.1 ± 5.5 vs. 13.3 ± 3.0 mg·hr/dl) than females ($n = 13$), and older subjects ($n = 13$) tended to have a greater postprandial increase in TRL triglyceride (1031 ± 159 vs. 609 ± 130 mg·hr/dl) and TRL apoB (17.4 ± 4.2 vs. 11.1 ± 3.1 mg·hr/dl) than younger subjects ($n = 9$). These differences did not reach statistical significance at the 0.05 level, however.

As reported previously for this group of subjects (11), postprandial rise in plasma triglyceride was positively ($r = 0.66$) and significantly ($P < 0.001$) correlated with fasting plasma triglyceride concentrations (Fig. 2A). Postprandial rise in TRL triglyceride was similarly correlated with fasting TRL triglyceride concentrations ($r = 0.58$, $P < 0.01$, Fig. 2B). However, the correlation between rise in TRL apoB concentration and fasting TRL apoB concentrations was not statistically significant (Fig. 2C).

The TRL fractions were further analyzed by separating their constituent apolipoproteins by polyacrylamide gradient gel electrophoresis. Eight protein bands were clearly

separated and identified for all 22 subjects. Gels from 3 subjects are shown in Fig. 3. Protein bands are identified at the left of each gel. A band (mol wt 55–60,000) was observed between the albumin and apoA-IV bands in all subjects. It appeared as a doublet in most samples. We identified this protein as being apoH (B₂-glycoprotein I) (19). The molecular weight of this protein by sedimentation equilibrium measurement is 43,000 (20), but since it is highly glycosylated, this protein appears to run above apoA-IV (mol wt 46,000). ApoH probably represents the human counterpart of the 59 kDa (apparent molecular mass) protein, apoA-V, which has been identified in rat chylomicrons (21). In some subjects a faint band was observed above the apoB-100 band, which may have represented apo[a]. (Apo[a] has been shown to increase in concentration in the TRL fraction after fat feeding (22).) Since the band was not consistently present in all subjects, we did not obtain data for this protein. It is apparent, however, that the present gel system could resolve this protein from apoB-100, and it therefore did not interfere with the quantitation of apoB-100. The lowest molecular band was identified as being comprised of the C apolipoproteins. ApoA-II also migrates within this band, but since this protein is a very minor component of TRL apolipoproteins (23), its contribution was taken to be negligible. The apoB-48 band was clearly resolved in all gels and was clearly visible in all TRL fractions. ApoB-48 was present in the TRL fraction of fasting plasma in all subjects, without exception. The five lanes on each gel (Fig. 3) contain (from left to right) the apolipoproteins of TRL fractions isolated at 0, 3, 6, 9, and 12 hr after the meal. The three subjects were chosen for presentation because they had different magnitudes of postprandial triglyceridemia, as depicted by their change in TRL triglyceride concentration, shown graphically at the bottom of each panel. As was typical for all subjects, the postprandial peak in TRL triglyceride concentration invariably corresponded with an increase in staining intensity of the apoB-100, apoB-48, apoE, and apoC protein bands.

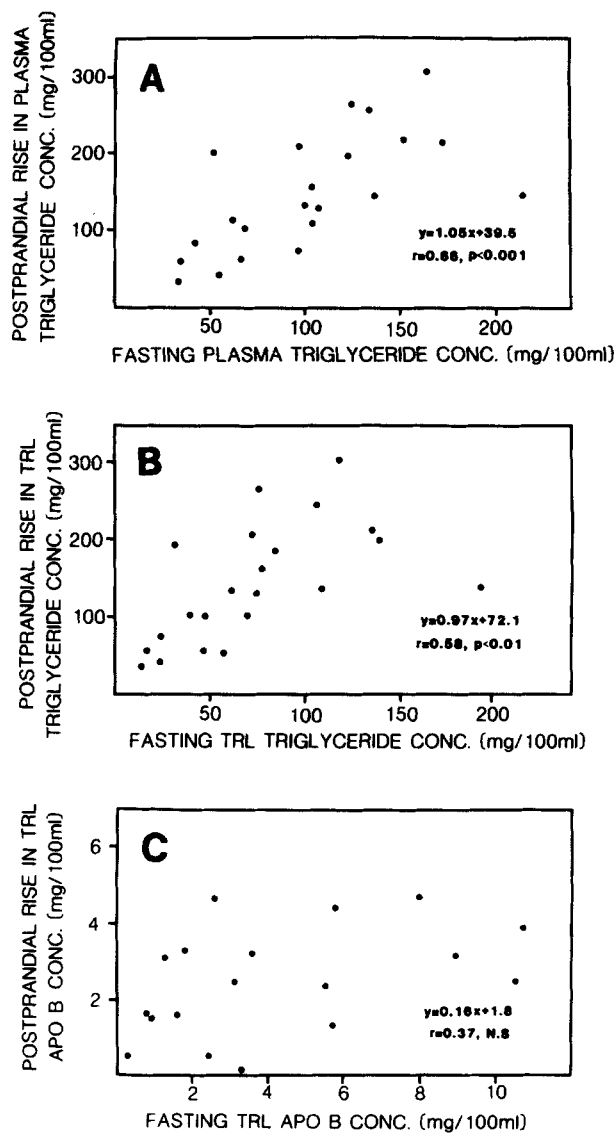


Fig. 2. Relationship between A) fasting plasma triglyceride concentrations and postprandial increases in plasma triglyceride concentration; B) fasting TRL triglyceride concentrations and postprandial increases in TRL triglyceride concentration; and C) fasting TRL apoB concentrations and postprandial increases in TRL apoB concentrations. A coefficient of correlation (r) is shown for each relationship, together with its level of statistical significance. Postprandial rise for each parameter was measured as the difference between zero-hour concentration and the maximum concentration observed postprandially.

In order to quantitate the changes observed in apolipoprotein composition, polyacrylamide gels were scanned in two dimensions with a laser densitometer. No attempt was made to use the scanning data to quantitate the apolipoproteins in absolute terms, since the individual apolipoproteins have differing chromogenicities (different capacities to retain stain) (17, 18). TRL apolipoprotein changes were instead measured in relative terms, as a change in the concentration of an apolipoprotein relative to its own concentration at a previous time point, or as a

change in the concentration of one apolipoprotein relative to another.

Postprandial changes in the concentration of TRL apoB-100 and apoB-48 were calculated from measurements of total TRL protein concentration and measurements of the staining intensity of the two apoB bands on the polyacrylamide gels. The concentration of the B apolipoproteins at each time point after the fat-rich meal was calculated as a percentage of their zero-hour (fasting) concentration, according to the formula given in the Methods section. As shown in **Fig. 4**, TRL apoB-100 and apoB-48 both increased significantly after the fat-rich meal. The maximum relative increase in apoB-48 ($561 \pm 45\%$, 3 hr postprandially) was considerably greater than the maximum relative increase in apoB-100 ($153 \pm 13\%$, 6 hr postprandially). In absolute terms (mg/dl), the concentration of TRL apoB-100 probably increased more than apoB-48, since the fasting concentration of TRL apoB-100 was probably 50–100 times greater than the fasting concentration of TRL apoB-48, based on inspection of the polyacrylamide gels (at zero-hour, the mean ratio of apoB-100/apoB-48 measured by densitometric scanning was 111.9 ± 13.4). Mean TRL apoB-100/apoB-48 ratios for the 22 subjects at time points after the fat meal were: 3 hr, 28.3 ± 2.5 ; 6 hr, 39.9 ± 2.2 ; 9 hr, 56.9 ± 7.5 ; 12 hr, 91.0 ± 15.2 (the 3 hr, 6 hr, and 9 hr ratios were significantly different from the zero-time value by paired t -test, $P < 0.001$).

To confirm the finding that TRL apoB-100 increased postprandially, apoB-100 was measured directly in eight subjects using an ELISA assay with a monoclonal antibody specific for apoB-100. The mean TRL apoB-100 concentrations were, 0 hr, 2.6 ± 1.0 mg/dl; 3 hr, 3.8 ± 1.1 mg/dl ($P < 0.001$); 6 hr, 4.2 ± 1.4 mg/dl ($P < 0.05$); 9 hr, 3.1 ± 1.2 mg/dl; 12 hr, 2.3 ± 1.1 mg/dl (the 3 hr and 6 hr concentrations were significantly different from zero time by paired t -test).

To determine whether the postprandial increase in apoB-48 was more pronounced in individuals with greater postprandial triglyceridemia, subjects were divided into three groups according to their magnitude of postprandial triglyceridemia, as measured by planimetry from their plasma triglyceride response curves. The mean magnitudes of postprandial triglyceridemia for the three groups were: group A ($n = 7$): 1008 ± 127 mg·hr/dl; group B ($n = 7$): 1924 ± 104 mg·hr/dl; group C ($n = 8$): 3158 ± 220 mg·hr/dl. Mean TRL triglyceride, TRL apoB, and TRL apoB-100/apoB-48 values for these three groups are shown in **Table 4**. Subjects in groups B and C had progressively greater TRL triglyceride and apoB concentrations at all time points, compared to subjects in group A. Significantly, however, no statistical difference was observed between the mean apoB-100/apoB-48 ratios. These data suggest that neither the preferential accumulation in the plasma of apoB-48-containing TRL from the intestine

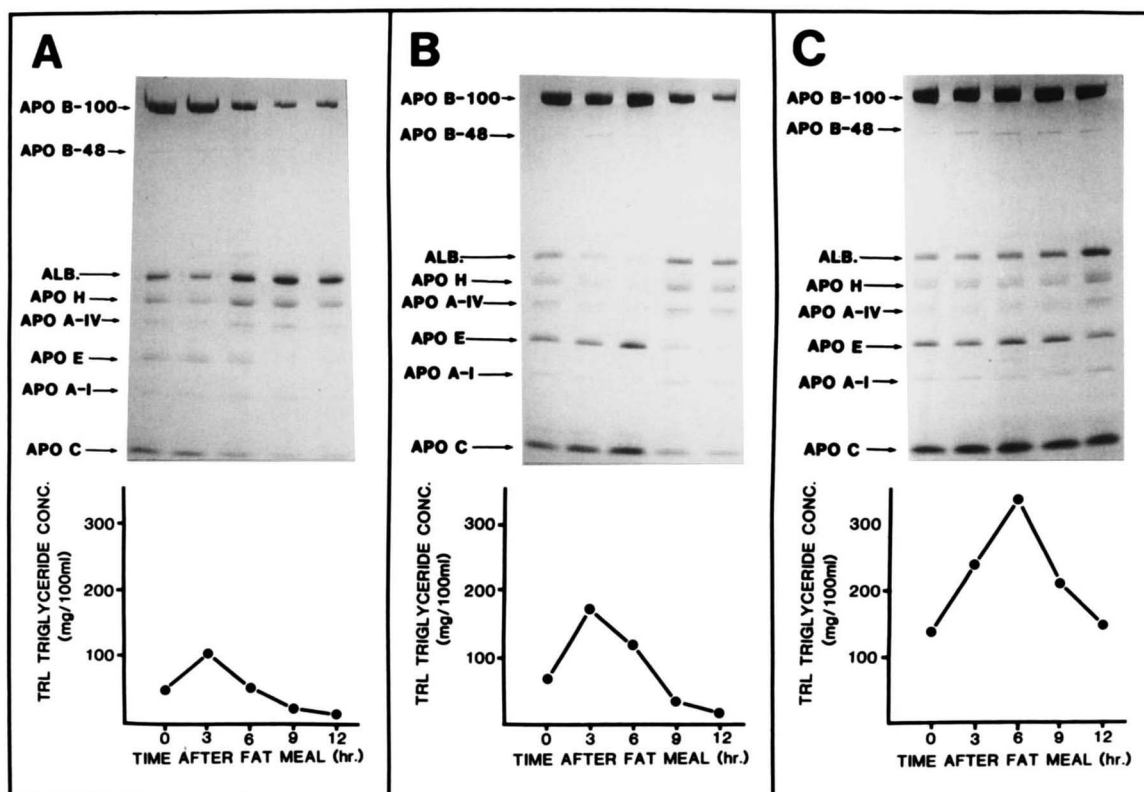


Fig. 3. Separation of TRL apolipoproteins by gradient (4–22.5%) polyacrylamide gel electrophoresis. The gels for three subjects, with increasing magnitude of postprandial triglyceridemia, are shown in the three panels. Postprandial changes in TRL triglyceride concentration are shown in the graphs at the bottom of each panel. The five lanes on each gel correspond (from left to right) to TRL fractions isolated at 0, 3, 6, 9, and 12 hr after the ingestion of the fat-rich meal. Panel A: Fractions from 25-year-old female; total postprandial plasma triglyceridemia = 894 mg · hr/dl. Panel B: Fractions from 65-year-old female; total postprandial plasma triglyceridemia = 1578 mg · hr/dl. Panel C: Fractions from 25-year-old male; total postprandial plasma triglyceridemia = 3224 mg · hr/dl. The apolipoprotein bands are identified at the left of each gel. Alb., albumin.

nor the preferential accumulation in plasma of apoB-100-containing TRL predominantly from the liver could account for the magnitude of postprandial triglyceridemia in these subjects.

The remaining TRL apolipoproteins were also quantitated from the polyacrylamide gels. Postprandial changes in these apolipoproteins are shown in **Fig. 5**. ApoE and apoC in TRL both increased significantly and then decreased. Albumin concentration in the TRL fraction increased at later time points and was significantly ($P < 0.05$) increased at 12 hr after the fat-rich meal. No mean significant postprandial change was observed in apoA-IV or apoH (data not shown). A postprandial increase in these apolipoproteins was observed in some subjects but not in others. We failed to observe the consistent increase in TRL apoA-IV, which has been observed by others after fat feeding (24, 25). Change in TRL apoA-I after the fat meal was also variable and no mean change was observed (**Fig. 5**). These results for apoA-I were confirmed by ELISA assay. Mean absolute TRL apoA-I concentrations ($n = 22$) were: 0 hr, 0.15 ± 0.02 mg/dl;

3 hr, 0.16 ± 0.02 mg/dl; 6 hr, 0.16 ± 0.02 mg/dl; 9 hr, 0.15 ± 0.02 mg/dl; 12 hr, 0.17 ± 0.02 mg/dl.

DISCUSSION

Several publications have documented the plasma apolipoprotein changes that occur when human subjects are fed a fat meal (26–32). In only two studies, to our knowledge, has apolipoprotein B been quantitated postprandially. Redgrave and Carlson (33) showed that VLDL apoB increased slightly in subjects with moderate postprandial triglyceridemia. Castro and Fielding (34) showed that plasma apolipoprotein B concentration did not change significantly in healthy volunteers fed a fat-rich meal; however, in this study only one postprandial time point was examined. In contrast, we have observed a small but significant decrease in plasma apolipoprotein B concentration after fat feeding (**Table 2**). TRL apoB concentration increased significantly (**Table 3**), and apoB in the $d > 1.006$ g/ml fraction of plasma decreased.

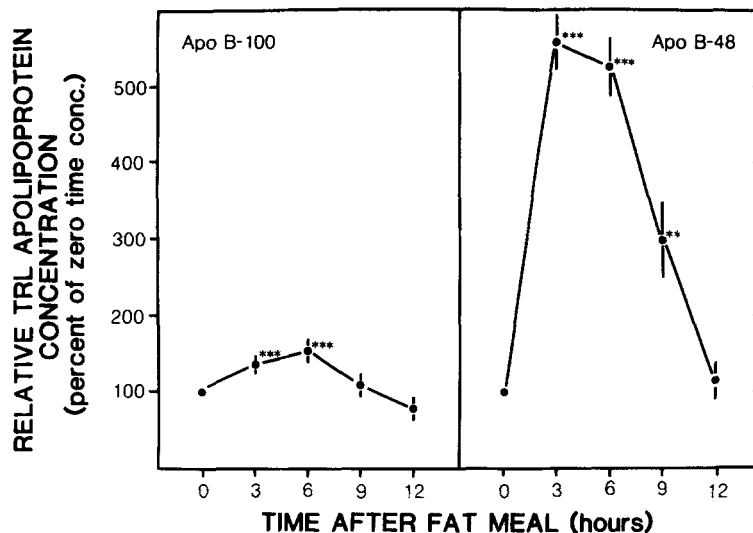


Fig. 4. Change in triglyceride-rich lipoprotein (TRL), apoB-100, and apoB-48 concentrations in subjects fed a fat-rich meal. Relative TRL apoB-100 and apoB-48 concentrations were calculated as a percentage of their zero-hour (fasting) concentrations. Quantitation of TRL apoB-100 and apoB-48 was achieved by densitometric scanning of the apoB-100 and apoB-48 bands of polyacrylamide gradient gels after electrophoretic separation of the TRL apolipoproteins (see Methods). Each point represents the mean \pm SEM for 22 subjects. Significantly different from zero time by paired *t*-test: ***P* < 0.01, ****P* < 0.001.

The postprandial increase in TRL apoB was due to a rise in both TRL apoB-100 and apoB-48 concentrations. Although some recent evidence suggests that the adult intestine can synthesize and secrete apoB-100 (7, 8), it appears that the apoB-100 in plasma is predominantly of hepatic origin. We have therefore concluded from our data that lipoproteins from both the liver and intestine appear to contribute to postprandial triglyceridemia. The postprandial increase in plasma TRL containing apoB-48 presumably reflects the increased secretion of chylomicrons by the intestine. The postprandial increase in plasma TRL containing apoB-100 is probably due to an

increase in secretion of TRL from the liver. Increased secretion of TRL by the liver in the fed state is supported by studies with perfused rat livers which have shown that livers isolated from fed rats secrete more triglyceride (35) and apoB (36) than livers from fasted rats. A postprandial increase in hepatic TRL secretion may represent the resecretion of dietary lipid that reaches the liver via the portal vein or via chylomicron transport. This possibility is supported by studies in HepG2 cells (37) and in perfused rat livers (38). A second possibility, that must also be considered, is that decreased catabolism of apoB-100 TRL could cause these lipoproteins to accumulate in

TABLE 4. Postprandial changes in TRL triglyceride, TRL apolipoprotein B, and TRL apoB-100/apoB-48 ratio in subjects grouped according to their magnitude of postprandial triglyceridemia^a

	Group	Time after Ingestion of Fat-Rich Meal				
		0 Hour ^b	3 Hour	6 Hour	9 Hour	12 Hour
TRL triglyceride (mg/dl)	A	33 \pm 6	84 \pm 11	71 \pm 18	52 \pm 11	24 \pm 5
	B	69 \pm 13*	217 \pm 25***	151 \pm 9**	85 \pm 13	47 \pm 10
	C	112 \pm 14***	285 \pm 17***	300 \pm 22***	178 \pm 34**	119 \pm 32*
TRL apolipoprotein B ^c (mg/dl)	A	1.8 \pm 0.4	2.9 \pm 0.6	2.2 \pm 0.6	1.1 \pm 0.2	0.5 \pm 0.1
	B	3.7 \pm 0.6*	5.7 \pm 0.8**	6.4 \pm 0.7***	3.2 \pm 0.6**	1.5 \pm 0.4*
	C	7.7 \pm 1.3**	9.6 \pm 1.7**	10.3 \pm 1.2***	9.9 \pm 2.2**	6.7 \pm 2.2*
TRL apoB-100/apoB-48 ^d	A	109 \pm 21	30 \pm 6	39 \pm 5	59 \pm 10	86 \pm 21
	B	120 \pm 26	25 \pm 3	46 \pm 9	56 \pm 20	80 \pm 24
	C	109 \pm 26	29 \pm 4	35 \pm 3	55 \pm 12	104 \pm 32

Values are means \pm SEM for seven subjects in group A, seven subjects in group B, and eight subjects in group C. Results are significantly different from group A by unpaired *t*-test: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

^aMagnitude of postprandial triglyceridemia was measured by calculating the area between curve and baseline, from the graph of plasma triglyceride concentration plotted against time.

^bZero-hr measurements were made on plasma obtained after a 14-hr overnight fast, before ingestion of the fat-rich meal.

^cTRL apoB was measured by ELISA assay using a polyclonal antibody that recognized both B-100 and B-48.

^dCalculated from the two-dimensional densitometric scans of polyacrylamide gradient gels.

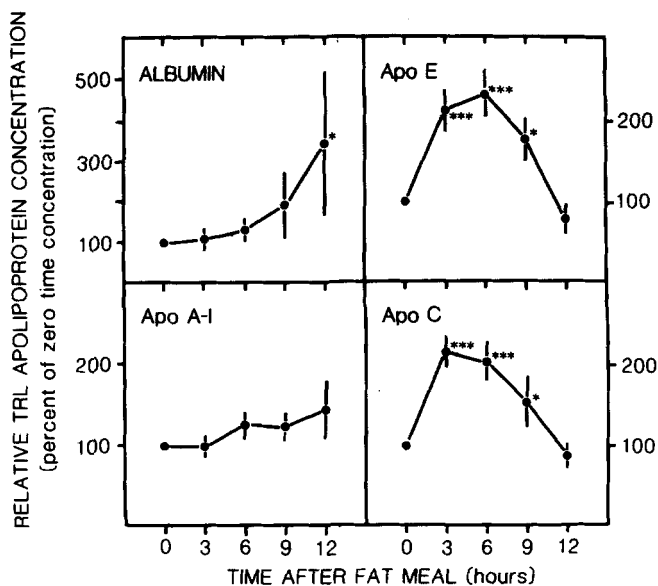


Fig. 5. Change in triglyceride-rich lipoprotein (TRL) apolipoprotein concentrations in subjects fed a fat-rich meal. Relative TRL apolipoprotein concentrations were calculated as a percentage of their zero-hour (fasting) concentrations. Each point represents the mean \pm SEM for 22 subjects. Significantly different from zero time by paired *t*-test: **P* < 0.05, ****P* < 0.001.

postprandial plasma. We have previously speculated (11) that the large postprandial influx of chylomicrons into the circulation after fat feeding could inhibit the conversion of VLDL to LDL. This is feasible, considering that lipoprotein lipase and apoC-II are required for both VLDL and chylomicron triglyceride hydrolysis. Saturation of the lipolytic process by intestinal TRL could cause the normal catabolism of hepatic TRL to be reduced, hence causing an accumulation of TRL containing apoB-100. The possible mechanisms responsible for the postprandial increase in plasma of apoB-100 TRL require further investigation.

The study of apoB-48 has been hampered by the lack of a quantitative assay for this protein. Since apoB-48 is structurally identical to a portion of apoB-100 (10, 39), antibodies that react to apoB-48 also react against apoB-100 (40). Immunochemical quantification of apoB-48 is therefore almost impossible. We have tried in our laboratory to quantitate apoB-48 by difference (41), using a polyclonal ELISA assay for measuring total apoB and a monoclonal assay specific for apoB-100. Accurate and reproducible measurement of apoB-48 by this method has proven difficult, however, due to the inaccuracy inherent in subtracting two large values to obtain a considerably smaller value. The quantification of apoB-48 in absolute terms, by scanning polyacrylamide gels, is also problematic, as demonstrated by Poapst, Uffelman, and Steiner (18). For these reasons, we have measured apoB-48 in relative terms only in the present study.

We found that apoB-48 was present in the TRL fraction of all 22 subjects after the 14-hr overnight fast. These results contrast with the original observations by Kane, Hardman, and Paulus (42), but support those of Cazzolato, Bittolo Bon, and Avogaro (43). Several explanations can be proposed for the presence of apoB-48 in the TRL fraction of fasting plasma. 1) Chylomicrons or VLDL containing apoB-48 are synthesized and secreted at a basal rate by the intestine in the fasting state (2); 2) a small fraction of chylomicron remnants containing apoB-48 escapes clearance by the liver and remains in the circulation; and 3) apoB-48 is synthesized by the liver in man, as described for the rat (44). Since the hepatic clearance of chylomicron remnants from the circulation appears to be a very rapid and efficient process (45, 46), and since there is now good evidence to suggest that human liver does not synthesize apoB-48 (6), it is most probable that a low basal rate of apoB-48 secretion from the intestine accounts for the small amount of this apoprotein in the plasma TRL fraction during fasting.

In the fasting state, we observed no correlation between the apoB-100/apoB-48 ratio and the TRL triglyceride concentration. Cazzolato et al. (43) have suggested that the presence of apoB-48 relative to apoB-100 increases as the mass of fasting plasma VLDL increases. In a second study, however, involving a larger number of subjects, the same relationship was not apparent (47). It is probable that only in certain severe cases of hypertriglyceridemia does the mass of apoB-48 in the TRL fraction reflect the degree of plasma triglyceride accumulation, as shown by Meng et al. (48). In the postprandial state, we also observed no correlation between the amount of apoB-48 relative to apoB-100 in the TRL fraction and the amount of TRL in plasma (Table 4). These results suggest that the magnitude of postprandial triglyceridemia is not determined solely by the extent of TRL secretion from the intestine, and support the concept that TRL from the liver make a significant contribution to the pool of triglyceride-containing lipoproteins that are present in the plasma after fat feeding.

The postprandial increase and then decrease in the B apolipoproteins of the TRL fraction (Table 3, Fig. 4) was associated with a rise then fall in the C and E apolipoproteins. As shown by others (31, 32), the postprandial increase in TRL apoC is due to the transfer of apoC-II and apoC-III from HDL during the early phase of fat absorption. During the later phases of fat absorption, apoC-II and apoC-III are recycled to HDL, and are also probably taken up by the liver on chylomicron remnants (31). ApoE also transfers between TRL and HDL in plasma, and the increase in apoE on TRL after alimentary lipemia, shown previously by Blum (49), is probably an important event leading to the uptake of TRL by specific hepatic receptors that recognize apoE.

Several previous studies (27, 31, 50) have documented the strong positive correlation between fasting plasma and TRL triglyceride levels and the magnitude of postprandial triglyceridemia (Fig. 2). In the present study, fasting TRL apoB concentrations were not, however, significantly correlated with postprandial TRL apoB concentrations (Fig. 2). These results suggest that those factors that control the plasma concentration of TRL triglyceride in the fasting state also strongly control the TRL triglyceride concentration in the postprandial state. This, however, does not seem to be the case for TRL apoB. Therefore, TRL catabolism is probably controlled more by the activity of lipoprotein lipase than by the activity of chylomicron-remnant receptors in the liver. This concept is supported by the recent finding that the magnitude of postprandial chylomicronemia is very strongly and inversely correlated with lipoprotein lipase activity (51).

An interesting and unexpected finding of the present study was the increase in the presence of albumin in the TRL fraction 12 hr after fat feeding. Albumin is not usually considered an apolipoprotein, though it is found associated with the triglyceride-rich lipoproteins (17). It is often considered a contaminating protein (52) and in many studies, steps are taken during lipoprotein isolation to free preparations of this protein. Albumin may, however, be a functionally important component of TRL, especially during lipolysis (53) when it acts as an acceptor of fatty acids generated by the action of lipoprotein lipase (54, 55). The present results suggest that the association of albumin with TRL is greater during the later phases of fat absorption when this fraction contains fewer nascent TRL and more particles that have been partially hydrolyzed. Further studies are required to establish the significance of the association of albumin to triglyceride-rich lipoproteins during lipolysis.

One interesting implication of the present findings is that fat feeding may affect LDL metabolism in a more direct fashion than previously appreciated. ApoB-100 TRL are the direct precursors of LDL in the circulation. Since the plasma concentration of apoB-100 TRL increases relatively soon after fat feeding (Fig. 4), one might hypothesize that the production of LDL is in turn increased. Paradoxically, however, we have found that LDL concentration does not increase postprandially. In fact, we have shown that LDL cholesterol (11) and apoB in the $d > 1.006$ g/ml fraction decrease after fat feeding. In all probability, this is due to increased hepatic uptake of apoB-100 TRL, mediated by the increased presence of apoE on these particles, or due to a slower conversion of VLDL to LDL caused by saturation of the lipolytic process (as discussed earlier). Clearly, much work is required to prove that these events actually take place; however, it is apparent that postprandial events can directly influence the plasma concentration of potentially atherogenic LDL.

In conclusion, the present study has shown that triglyceride-rich lipoproteins from the liver appear to make a significant contribution to postprandial triglyceridemia. Thus, the magnitude of plasma triglyceride increase after fat feeding reflects the balance between secretion and catabolism of both intestinal and hepatic triglyceride-rich lipoproteins. ■■

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