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Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans

(chylomicrons/very low density lipoproteins/cholesterol)

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ABSTRACT **The concentration of triglyceride-rich lipoproteins containing apolipoprotein (apo) B-48 (chylomicrons) and apo B-100 (very low density lipoproteins) was measured in blood plasma of healthy young men after an ordinary meal containing one-third of daily energy and fat. Plasma obtained in the postabsorptive state and at intervals up to 12 hr after the meal was subjected to immunoaffinity chromatography against a monoclonal antibody to apo B-100 that does not bind apo B-48 and a minor fraction of apo B-100 rich in apo E. Measurements of the concentrations of components of the total and unbound triglyceride-rich lipoproteins separated from plasma by ultracentrifugation showed that about 80% of the increase in lipoprotein particle number was in very low density lipoproteins containing apo B-100 and only 20% was in chylomicrons containing apo B-48 that carry dietary fat from the intestine. The maximal increments and the average concentrations of apo B-48 and B-100 during the 12 hr were highly correlated (r^2 = 0.80), suggesting that preferential clearance of chylomicron triglycerides by lipoprotein lipase leads to accumulation of hepatogenous very low density lipoproteins during the alimentary period. The composition of the bulk of very low density lipoproteins that were bound to the monoclonal antibody changed little and these particles contained about 90% of the cholesterol and most of the apo E that accumulated in triglyceride-rich lipoproteins. The predominant accumulation of very low density lipoprotein rather than chylomicron particles after ingestion of ordinary meals is relevant to the potential atherogenicity of postprandial lipoproteins.**

Most individuals spend 12 hr or more daily in an alimentary (postprandial) state during which dynamic remodeling of lipoprotein particles occurs. After the first meal of the day, the typical pattern of meal eating is likely to sustain a lipemic state throughout the day since the peak in triglyceride response is usually 3–4 hr after the meal (1–5). The increase in plasma triglycerides after a meal is derived from exogenous (dietary) and endogenous (hepatic) sources, as indicated by increased levels of apolipoprotein (apo) B-100 and B-48 in triglyceride-rich lipoproteins (TRL) (2–4). In humans, apo B-48 is derived from secretion of chylomicrons from the small intestine, whereas apo B-100 is predominantly associated with TRL made in the liver (6).

Apo B-containing lipoproteins have been associated with risk of cardiovascular disease. However, the interrelationships among the various apo B-containing fractions and risk are complex. Remnants generated from chylomicrons as well as very low density lipoproteins (VLDL) are cleared by receptor-mediated processes in the liver (7). Since cholesteryl esters are transferred to TRL during the postprandial period, hepatic remnant uptake, in addition to delivering diet-derived lipids to the liver, is involved in reverse-cholesterol transport, which is thought to be an antiatherogenic process (8, 9). Alternatively, the TRL generated postprandially can potentially deposit cholesterol in the vessel wall; thus, prolonging their presence in blood may increase atherogenic risk (10). Both of these processes are probably important and the balance between them may determine the contribution of postprandial lipemia to cardiovascular disease risk. A major limitation in understanding this relationship has been the ability to quantify the relative contributions of the intestine and liver to the increase in TRL during alimentary lipemia.

Retinyl esters have been used by many investigators to measure the intestinal contribution to human alimentary lipemia by incorporating retinol or retinyl palmitate into a fat-containing meal and following the appearance of retinyl ester in the plasma (11–14). The accuracy of this method has been questioned, however, because the peak in plasma retinyl ester concentration does not correspond with the peak in triglyceride concentration and because of experimental evidence that retinyl esters are transferred from chylomicrons to other lipoprotein fractions (14). This transfer of retinyl esters indicates that the presence of retinyl esters in plasma does not always reflect the presence of intestinally derived lipoproteins. Although Krasinski *et al.* (14) have suggested that measurement of apo B-48 is the best method to determine the concentration of chylomicrons, accurate measurement of apo B-48 has been complicated by its low concentration in plasma and TRL, relative to the concentration of apo B-100.

We have used a monoclonal antibody to separate apo B-48-containing TRL from most apo B-100-containing lipoproteins so that the concentration of apo B-48 as well as that of apo B-100 in fasting and postprandial plasma samples could be determined.

METHODS AND MATERIALS

Subjects. Blood samples were obtained from seven healthy men participating in an ongoing study of postprandial lipemia. Average body weight among the subjects was 74.6 ± 2.4 kg (SE) and average height was 178.9 ± 2.8 cm. The average energy content of the test meal [1015 ± 40 kcal (1 kcal = 4.18 kJ); 38% from fat, 46% from carbohydrate, 16% from protein] provided one-third of daily caloric need. Average cholesterol content of the meals was 141 mg. For all test meals the ratio of polyunsaturated to saturated fatty acids was 0.8.

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Abbreviations: apo, apolipoprotein; TRL, triglyceride-rich lipoprotein(s); VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s).

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Preparation of Samples. JI-H antibody bound to CNBr-activated Sepharose-4B was used to prepare columns, as described (15). Plasma was applied to the column (15) and the unbound fraction was eluted at a rate of 15 ml/hr with saline/EDTA, pH 7.4 [150 mM NaCl/1.3 mM EDTA containing NaN_3 (0.02 mg/ml) and benzamidine (0.3 mg/ml)]. Bound material was washed from the column with 3 M NaSCN (pH 7.4) containing bovine serum albumin (1 mg/ml) and discarded, and the column was washed again with saline/EDTA. The unbound fraction was concentrated overnight at 4°C in a dialysis/concentrator (Bio-Molecular Dynamics, Beaverton, OR) containing saline/EDTA, using a dialysis membrane with a molecular weight cut-off of 10,000. The concentrated material was centrifuged in a Beckman ultracentrifuge (40.3 rotor) at 35,000 rpm, 12°C for 18 hr, and the $\rho < 1.006$ g/ml fraction was removed. The $\rho < 1.006$ g/ml fraction was further concentrated, as needed, by membrane filtration in a microconcentrator with a molecular weight cut-off of 10,000 (Centricon-10; Amicon). This material is designated unbound TRL. The total TRL fraction ($\rho < 1.006$ g/ml) from plasma was obtained by ultracentrifugation as above. In two additional subjects, two fractions of TRL with Svedberg flotation rates of 20–100 and >100 were obtained by density gradient ultracentrifugation (16).

TRL were delipidated overnight at −20°C with 20 vol of ethanol/ether (3:1). The samples were centrifuged at 1500 rpm (380 × *g*) for 20 min at −10°C. The pellet was washed with ether and the samples were centrifuged again. After removing the ether, the moist pellet was solubilized in sample buffer (0.125 M Tris, pH 6.6 in 10% glycerol, containing SDS (30 mg/ml), dithiothreitol (15 mg/ml), mercaptoacetate (10 mg/ml), and bromphenol blue (0.025 mg/ml) and the samples were heated for 3 min at 90°C.

Gel Electrophoresis. Electrophoresis (4–20 μg of protein per sample) was carried out in 3–10% linear polyacrylamide slab gels according to the method of Laemmli (17) at a constant current of 15–25 mA per gel for 4–5 hr in a vertical gel apparatus (Hoefer; model SE600). The gels were stained overnight in 0.25% Coomassie R-250 (Sigma) in methanol/water/acetic acid (5:5:1), destained for 7–8 hr in methanol/water/acetic acid (5:5:1), and dried overnight. Each lane was scanned in a densitometer (Clifford, Natick, MA; model 445), and the area of each peak was calculated and converted to μg of protein based on the chromogenicity of apo B-48 and apo B-100.

Quantification of Apo B-100 and Apo B-48. To prepare pure apo B-48, a fraction of VLDL rich in apo B-48 from a subject with familial dysbetalipoproteinemia was obtained by sequential immunoaffinity chromatography on columns containing monoclonal antibody JI-H and a monoclonal antibody (4G3) against a C-terminal domain of apo B-100. The unbound lipoprotein was concentrated, delipidated as above, and dissolved in 150 mM NaCl/10 mM sodium phosphate, pH 7.2 (PBS), in 10% glycerol containing SDS (20 mg/ml) and EDTA (0.1 mg/ml) and applied to a 1.2 × 90 cm column of Ultrogel AC-22 (Pharmacia). The apo B-48 peak was eluted with PBS containing SDS (1.0 mg/ml) and EDTA (0.1 mg/ml). Delipidated human low density lipoprotein (LDL) was used similarly to obtain apo B-100 by elution from the Ultrogel column. The purity of apo B-48 and apo B-100 was verified by SDS gel electrophoresis and their mass was determined by the method of Lowry *et al.* (18). Standard curves based on dye uptake of isolated apo B-100 and apo B-48 are shown in Fig. 1. The regression coefficients for human apo B-100 and apo B-48 do not differ statistically, as reported previously for the rat proteins (19).

Other Analyses. Total cholesterol and triglycerides were determined by enzymatic assays (20, 21) and apo E was determined by radioimmunoassay (22).

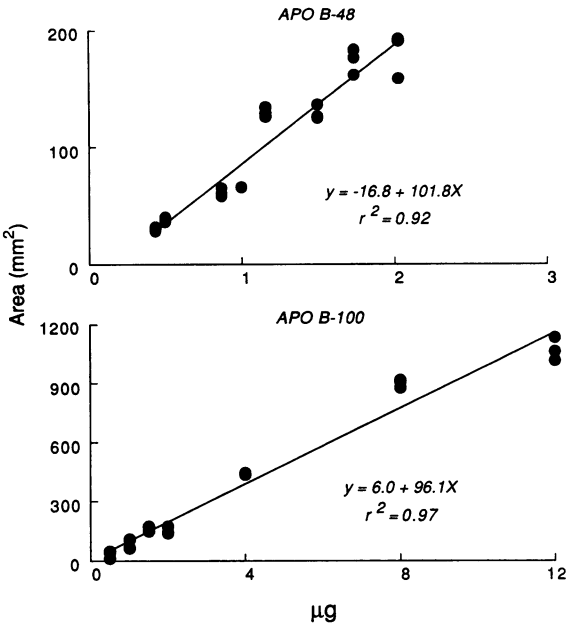

FIG. 1. Densitometric areas of Coomassie blue-stained apo B-48 and apo B-100 separated by SDS gel electrophoresis. Samples containing known amounts of protein were applied in 100 μl of sample buffer.

Statistical Analysis. Data were subjected to a repeated measures ANOVA to determine significant changes with time. Correlations between the concentration of apo B-48, apo B-100, apo E, cholesterol and triglycerides in plasma, TRL, and unbound TRL were estimated by linear regression analysis.

RESULTS

Figs. 2–5 show the average changes in concentrations of triglycerides, cholesterol, apo B-100, apo B-48, and apo E with time and the results of the repeated measures ANOVA. Plasma triglycerides increased significantly at 3 hr (Fig. 2) but plasma cholesterol concentration did not change (Fig. 3). In total TRL and unbound TRL the concentrations of triglycerides, cholesterol, apo B-100, apo B-48, and apo E increased significantly after the meal and fell below fasting levels by 9 and 12 hr (Figs. 2–5). In one of the seven subjects, no apo B-48 (<0.04 mg/dl) could be detected at any time. In six of the seven, measurable amounts of apo B-48 were present in the postabsorptive state, but in only one of these could the protein be detected at 9 hr. The average total plasma concentration of apo E was significantly higher at 3 hr (3.44

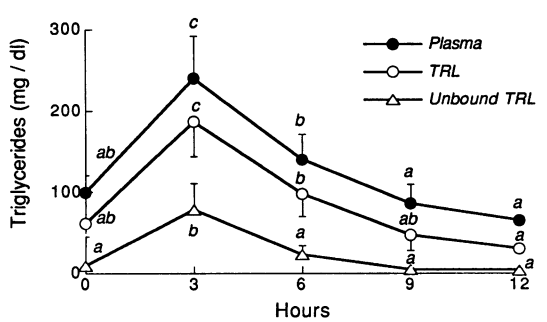

FIG. 2. Mean concentration of triglycerides in plasma, TRL, and unbound TRL in seven men who consumed a fat-rich meal (one value missing at 12 hr). Bars indicate 1 SE. Values for each component that differ significantly ($P < 0.05$) are denoted by different letters.

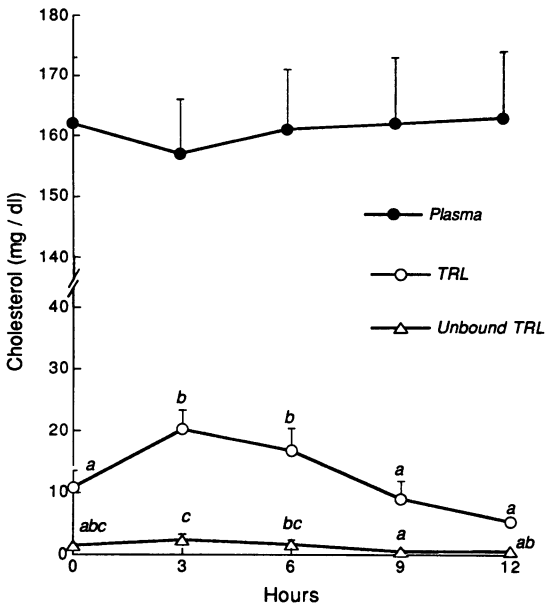

FIG. 3. Mean concentration of cholesterol in plasma, TRL, and unbound TRL in seven men who consumed a fat-rich meal. Symbols and missing value as in Fig. 2.

mg/dl) than at 6, 9, or 12 hr (3.07, 2.67, and 2.62 mg/dl, respectively).

For several subjects the difference between cholesterol, triglycerides, and apo B-100 concentrations in total TRL and unbound TRL was calculated to estimate the amount of cholesterol or triglycerides associated with the bulk of TRL that contained apo B-100 but no apo B-48 (Table 1). The concentrations of all three components increased significantly at 3 hr and that of cholesterol and apo B-100 remained significantly higher at 6 hr. As shown in Figs. 1 and 2, about 50% of the increase in TRL-triglycerides was in the bound fraction, whereas >90% of the increase in TRL-cholesterol was in this fraction. The composition of lipoproteins in the bound fraction, as deduced from the ratio of triglycerides to apo B-100 and of cholesterol to triglycerides (Table 1), changed little except for a marginal increase in the former

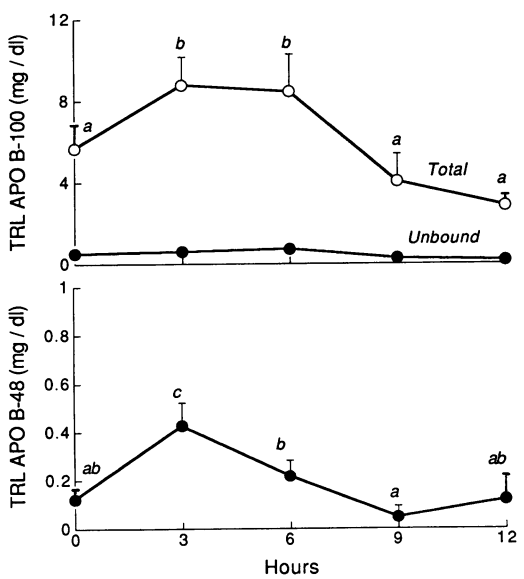

FIG. 4. Concentration of apo B-100 in TRL and of apo B-100 and apo B-48 in unbound TRL in seven men who consumed a fat-rich meal. Symbols and missing value as in Fig. 2.

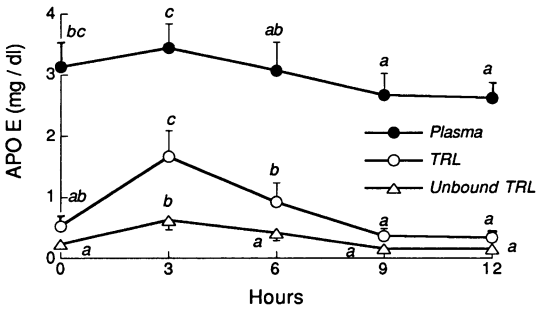

FIG. 5. Concentration of apo E in plasma, TRL, and unbound TRL in seven men who consumed a fat-rich meal. Symbols and missing value as in Fig. 2.

ratio at 3 hr ($P = 0.15$). This result was verified in two subjects whose plasma obtained at fasting and 3 hr after the test meal was separated by density gradient ultracentrifugation. About 80% of the increase in apo B-100 concentration within TRL occurred in particles with Svedberg flotation rates between 20 and 100 and only about 20% occurred in larger particles (data not shown).

The average concentration of TRL-apo B-48 during the 12-hr period was well correlated with that of TRL-apo B-100, TRL-triglycerides, and TRL-cholesterol, and TRL-apo B-100 concentration was also well correlated with that of TRL-triglycerides and TRL-cholesterol (Table 2). Likewise, the increases in concentration of apo B-48 and B-100 at the peak of alimentary lipemia were highly correlated and each of these was also well correlated with the increase in TRL-triglyceride concentration (Fig. 6).

DISCUSSION

In the current study, in which test meals containing normally consumed foodstuffs were fed rather than a liquid formula, we observed that the concentrations of apo B-100, apo B-48, and apo E, as well as triglycerides and cholesterol, increased substantially in TRL. The concentration of apo B-48 was significantly higher 3 hr after the meal, whereas those of apo B-100 as well as triglycerides and cholesterol were significantly higher after 3 and 6 hr. Cohn *et al.* (3) likewise have observed increases in apo B-48 and apo B-100 after consumption of a liquid formula that provided 53% of energy from fat, but they were unable to quantify the absolute changes in concentration of apo B-48. The increases in apo B-100 concentration in TRL that we observed after fat feeding are comparable to those reported by Genest *et al.* (2) and Cohn *et al.* (3). Additionally, we observed that the postprandial responses of apo B-100 and apo B-48 are correlated with those of TRL triglycerides, as suggested by others (12, 13, 23). Our data, and those of others (2–4), indicate that increases in the concentration of triglyceride-rich particles containing apo B-100 as well as those containing apo B-48 must be considered in any evaluation of the association of postprandial lipemia with atherogenic risk.

Other investigators have reported that the plasma concentration of apo B either does not change or even falls after a meal (3, 8); however, measurement of apo B in whole plasma is inadequate to detect the differences in apo B concentration that occur within TRL. The average increase within TRL in the concentration of apo B-48 was 0.30 mg/dl (11.4 nmol/liter), whereas that of apo B-100 was 3.1 mg/dl (57 nmol/liter). Although the increase in apo B-48 represented a 3.5-fold difference in concentration as compared with a 1.6-fold increase in apo B-100, apo B-100 accounted for about 80% of the increase in lipoprotein particles in TRL. This increase in apo B-100 reflects a higher concentration of VLDL of relatively constant composition.

Table 1. Concentration of components of triglyceride-rich lipoproteins bound to monoclonal antibody JI-H during alimentary lipemia in healthy young men

Time, hr	Apo B-100, mg/dl	TG, mg/dl	CH, mg/dl	TG/apo B-100	CH/TG
0	5.15 ± 1.04[a]	36.8 ± 6.4[a]	5.9 ± 0.76[a]	9.9 ± 0.59	0.16 ± 0.01
3	8.16 ± 1.33[b]	95.2 ± 25.8[b]	15.3 ± 2.8[b]	13.1 ± 1.0	0.17 ± 0.02
6	7.70 ± 1.74[b]	52.8 ± 10.6[a]	11.4 ± 2.7[b]	8.8 ± 2.1	0.22 ± 0.04
9	3.75 ± 1.30[a]	23.2 ± 2.6[a]	4.9 ± 1.0[a]	9.2 ± 1.9	0.22 ± 0.04
12	2.75 ± 5.00[a]	27.7 ± 6.7[a]	4.4 ± 0.90[a]	10.8 ± 2.1	0.17 ± 0.02

Values are mean ± SE; those with different superscripts differ significantly ($P < 0.05$). TG, triglycerides; CH, cholesterol. n = 7, 4, and 5 for apo B-100, triglycerides, and cholesterol, respectively.

The alimentary increase in TRL apo B-100 could reflect delayed clearance of VLDL particles, enhanced hepatic secretion, or both. Enhanced secretion of VLDL-triglycerides might occur as a result of augmented hepatic uptake of fatty acids derived from chylomicron triglycerides. In our subjects fed a mixed meal, any such increment would, however, be offset by reduced delivery to the liver of fatty acids produced by hydrolysis of triglycerides in adipose tissue. Furthermore, the remarkably close correlation between the increment in concentrations of TRL apo B-48 and apo B-100 after the meal indicates that reduced efficiency of chylomicron particle clearance is closely coupled to accumulation of VLDL particles. It is unlikely that hepatic VLDL secretion would be stimulated by less efficient removal of chylomicron lipid. Rather, this correlation, together with the relatively constant size of the VLDL particles, strongly suggests that hepatogenous VLDL accumulate as a result of preferential clearance of chylomicron triglycerides by lipoprotein lipase (24). This interpretation is supported by observations of Potts *et al.* (25), who have reported that after a fat-rich meal the clearance of chylomicron-triglycerides into human subcutaneous adipose tissue is enhanced, whereas that of VLDL-triglycerides is reduced, and by those of Robins *et al.* (26), who found in rats that accumulation of endogenous triglycerides in plasma is inversely correlated with the rate of clearance of intravenously administered triglyceride emulsions. Delayed clearance of chylomicron particles, as evidently occurs in many hypertriglyceridemic states, may thus contribute to elevations of apo B-100 in TRL. Given the evident rapid metabolism of chylomicron triglycerides (27, 28), it seems likely, as proposed recently by Berr (29), that particles containing apo B-48 in plasma after a fat-rich meal represent mainly chylomicron remnants, clearance of which is saturated for several hours. Although hydrolysis of VLDL-triglycerides evidently is impeded during alimentary lipemia, it is notable that VLDL particles in the bound fraction from the immunoaffinity column became greatly enriched in apo E at the peak of lipemia. The calculated ratio of apo E to apo B-100 in bound VLDL increased from 0.057 in the postabsorptive state to 0.127 after 3 hr. In this respect, these particles also became more "remnant-like" even though they retained affinity for monoclonal antibody JI-H.

We found that >90% of the increase in TRL-cholesterol at the peak of lipemia was associated with bound VLDL (i.e., exclusively in particles containing apo B-100). We thus conclude that dietary cholesterol makes only a minor contribution to the increment in TRL-cholesterol after fat ingestion. This is not unexpected, as ordinary meals have at least 100-fold more triglyceride mass than cholesterol. Other studies have shown, with a larger dietary fat load, that the increment in TRL-cholesterol occurs concomitantly with a fall in LDL-cholesterol (30). It can thus be proposed that the prolonged residence time of VLDL during alimentary lipemia leads to augmented transfer of cholesterol from other lipoproteins, mainly LDL.

The increment of apo B-100 in TRL observed here may differ with the amount and composition of dietary fat. Consumption of a fat load containing saturated fatty acids re-

Table 2. Correlations among concentrations of apo B-48, apo B-100, triglycerides, and cholesterol in lipoproteins of $\rho < 1.006$ g/ml during alimentary lipemia in healthy young men

Correlation	r^2
Apo B-48 vs. TG	0.82
Apo B-48 vs. CH	0.86
Apo B-100 vs. TG	0.94
Apo B-100 vs. CH	0.90
Apo B-48 vs. apo B-100	0.80
CH vs. TG	0.97

Mean concentrations of each analyte found in each subject during the 12 hr of study were used in this analysis. All values are significant at $P < 0.01$ (n = 7). TG, triglycerides; CH, cholesterol.

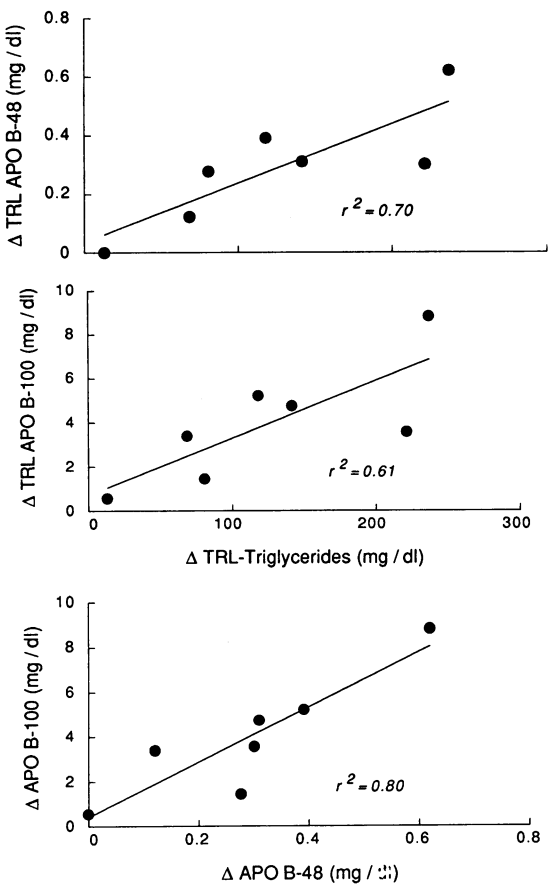

FIG. 6. Relationships between increments in apo B-100, apo B-48, and triglycerides in TRL in seven men after a fat-rich meal. Increases were calculated as the difference between the initial (postabsorptive) level and the highest postprandial level at 3 or 6 hr. All correlations shown are significant ($P < 0.05$).

portedly causes a greater alimentary triglyceridemic response than a comparable load containing mainly polyunsaturated fatty acids (12), perhaps reflecting slower hydrolysis of saturated fatty acids by lipoprotein lipase. This greater response would be expected to lead to an increased accumulation of VLDL, but whether such an increase contributes to the cholesterol-elevating property of saturated fatty acids is unclear.

Delayed clearance of chylomicron remnants has been proposed to be a possible atherogenic risk factor (10) and persistent elevation of plasma triglyceride levels after a fat-rich meal has recently been reported to be independently predictive of coronary artery stenosis in a case-control study (31). Nestel *et al.* (32) have reported that hypertriglyceridemic subjects have delayed clearance of chylomicron particles after a fat-rich meal. In our subjects without abnormalities of lipoprotein metabolism, intestinally derived lipoproteins produced during alimentary lipemia, as measured by concentration of apo B-48, generally became undetectable 9 hr after the meal and are thus virtually removed from plasma after 6–9 hr. Given the persistence of these particles for at least 6 hr after an ordinary meal containing one-third of daily energy, most individuals would normally have eaten a second meal before chylomicron levels returned to baseline. As apo B-48 and apo B-100 concentrations probably remain elevated above postabsorptive levels throughout most of the day, it will be important to understand the determinants of the magnitude of these responses.

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