

Remnant-like particle cholesterol and triglyceride levels of hypertriglyceridemic patients in the fed and fasted state

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Abstract Potentially atherogenic triglyceride-rich lipoprotein (TRL) remnants can be isolated and quantitated as remnant-like particles (RLP), using an immunoaffinity gel containing specific anti-human apolipoprotein A-I (apoA-I) and apoB-100 monoclonal antibodies. The aim of the present study was to determine the relationship between postprandial changes in RLP levels and changes in total serum triglyceride (TG) in patients with different forms of hypertriglyceridemia (HTG). Three groups of patients were selected, having similarly elevated serum TG levels: a) HTG with TRL remnant accumulation (i.e., type III patients, n = 15, TG: 3.8 ± 0.2 mM), b) HTG with increased LDL (i.e., type IIb patients, n = 15, TG: 3.7 ± 0.2 mM), and c) HTG without evidence of remnant or LDL accumulation (i.e., type IV patients, n = 15, TG: 3.9 ± 0.3 mM). Ingestion of a 45-g fat meal caused a significant increase in serum TG (30–50%) in all patients. Mean serum TG levels of the three groups were not significantly different at 4 or 6 h after the meal. RLP cholesterol (C) and TG levels increased after the meal in all patients, but these postprandial increases were also not significantly different among groups. Type III patients had significantly higher ($P < 0.01$) levels of RLP-C and RLP-apoE in the fasted and fed state, and also had significantly higher RLP-C-to-serum TG ratios ($P < 0.001$) compared with the other groups. These results indicate that 1) RLP-C and RLP-TG levels are significantly increased in the fed versus fasted state in patients with elevated fasting TG levels; 2) patients with different forms of HTG, but similar TG levels, have similar postprandial increases in RLP-C and RLP-TG; and 3) type III patients have significantly elevated levels of RLP-C and RLP-apoE in both the fed and fasted state.—Marcoux, C., P. N. Hopkins, T. Wang, E. T. Leary, K. Nakajima, J. Davignon, and J. S. Cohn. Remnant-like particle cholesterol and triglyceride levels of hypertriglyceridemic patients in the fed and fasted state. *J. Lipid Res.* 2000. 41: 1428–1436.

Supplementary key words cholesterol • atherosclerosis • type III hyperlipoproteinemia

The accurate detection and quantification of potentially atherogenic triglyceride-rich lipoprotein (TRL) remnants

has proven to be difficult, because these lipoproteins are cleared rapidly from the blood circulation and are subsequently found at relatively low plasma concentrations. In addition, no single biochemical method can adequately identify and/or separate all remnant lipoproteins, because of their heterogeneity in size, density, and composition (1). A more quantitative and clinically applicable assay has been developed, whereby remnant-like particles (RLP) are separated from plasma using an immunoaffinity gel containing an anti-human apolipoprotein A-I (apoA-I) antibody together with a specific apoB-100 monoclonal antibody (JI-H) (2, 3). Patients with coronary artery disease (CAD) have significantly increased plasma concentrations of RLP cholesterol (RLP-C) (2–4). Elevated RLP levels are in turn associated with impaired endothelium-dependent vasorelaxation (5, 6) and are predictive of future coronary events in patients with CAD (7).

In the fasted state, a strong positive correlation exists between RLP-C and total plasma triglyceride (TG) concentrations (2, 3, 8), and patients with hypertriglyceridemia (HTG) invariably have increased fasting levels of RLP (9). HTG patients with type III hyperlipoproteinemia (HLP), who have a pronounced increase in circulating remnant lipoproteins (i.e., beta very low density lipoprotein, β -VLDL) (10), have significantly increased fasting RLP levels (11, 12) and their ratios of RLP-C to plasma TG are significantly greater than those of patients with other forms of HTG (12, 13). In all these studies, plasma RLP levels have been determined in plasma or serum obtained after an overnight fast, and limited information is available concerning plasma RLP concentrations in the fed state. Furthermore, it is unclear whether patients with different

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; HDL, high density lipoprotein; HLP, hyperlipoproteinemia; HTG, hypertriglyceridemia; LDL, low density lipoprotein; RLP, remnant-like particle; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

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forms of HTG have similar postprandial changes in RLP. The aim of the present study was therefore to investigate the relationship between postprandial changes in RLP levels and changes in total serum TG in patients with different forms of HTG. Three groups of HTG patients have been investigated, who were selected so that they had similarly elevated fasting TG levels: *a*) HTG with fasting plasma TRL remnant accumulation (i.e., type III HLP patients), *b*) HTG with increased fasting levels of LDL (i.e., type IIb HLP patients), and *c*) HTG without evidence of fasting remnant or LDL accumulation (i.e., type IV HLP patients).

MATERIALS AND METHODS

Subjects

HTG patients were recruited from either the Lipid Clinic of the Clinical Research Institute of Montreal or the Clinic of the Department of Cardiovascular Genetics Research at the University of Utah in Salt Lake City. The clinical study protocol was approved by local institutional review committees and all patients gave their informed consent to participate. Male or female patients were selected if they were 18 years of age or older, and if they had a history of HTG. Patients were selected if they had not been taking lipid-lowering medications for at least 6 weeks before the study. They were excluded if they had had a myocardial infarction, unstable angina, coronary artery bypass grafting, or angioplasty within the 6 months before recruitment. A total of 77 patients were given the fat-containing meal. Patients were then selected for the present analysis, if they had a fasting plasma TG concentration between 2.3 and 9.0 mM (200–800 mg/dL) on the morning of the experiment. A total of 61 patients met this criterion. They were classified as having *a*) type III HLP, if they had an apoE 2/2 phenotype and the presence of β -VLDL on agarose gel electrophoresis, *2*) type IIb HLP, if they had an LDL cholesterol level greater than 4.2 mM (160 mg/dL) and no evidence of β -VLDL, or *3*) type IV HLP, if they had an LDL cholesterol less than 4.2 mM and no evidence of β -VLDL. Fifteen patients were then arbitrarily selected from each HLP group (a total of 45 patients), in such a way that the final three HLP groups had similar mean ages and TG levels.

Fat-rich meal protocol and blood sampling

After a 12-h overnight fast, patients were given a fat-rich breakfast consisting of one sausage McMuffin with egg and cheese, one serving of hash browns, and 8 fl. oz of whole milk. The total fat

content of the meal was 45 g. Venous blood samples were obtained in the fasted state (before the meal at 0 h) and in the fed state (at 4, 6, and 8 h postprandially). Blood was drawn into SST Vacutainer tubes (Becton Dickinson, Lincoln Park, NJ) and was allowed to clot for 30 min at room temperature. Tubes were centrifuged at 2,500 rpm for 10 to 15 min at 4°C to separate serum from clotted blood. Serum samples from both study sites were transferred to storage tubes and were sent refrigerated (but unfrozen) to Pacific Biometrics (Seattle, WA) for lipid and lipoprotein analyses, and for separation and analysis of RLP fractions. Serum samples from patients studied in Montreal were also stored at 4°C at the Clinical Research Institute of Montreal for analysis of RLP apolipoproteins.

Lipid and lipoprotein analyses

RLP were isolated from fresh serum with RLP cholesterol assay kits (JIMRO-II; Japan Immunoresearch Laboratories, Japan). According to the manufacturer instructions, 5 μ L of serum was added to 300 μ L of gel suspension containing antibodies. After gentle mixing for 2 h at room temperature with a vertical magnetic bead oscillator (RLP Mixer J-100A; Photal, Otsuka Electronics, Japan), the gel was allowed to settle for 15 min and the supernatant, containing the unbound RLP, was analyzed for cholesterol and triglyceride on a Cobas MIRA S chemistry analyzer (Roche Analytical Instruments, Nutley, NJ). The within-run imprecision (coefficient of variation) for the measurement of RLP cholesterol was 6.1 and 3.5% for a low and high control, respectively; the between-run imprecision was 10.2 and 5.6%, respectively (3). Total serum cholesterol and triglyceride concentrations were measured by Centers for Disease Control (CDC)-standardized enzymatic methods. For fasted (0 h) samples only, HDL cholesterol was measured after precipitation of non-HDL lipoproteins by dextran sulfate (M_r 50,000)- Ca^{2+} . VLDL ($d < 1.006$ g/mL) and non-VLDL lipoproteins ($d > 1.006$ g/mL) were separated by ultracentrifugation in a Beckman (Fullerton, CA) Ti 42.2 rotor (100,000 g for 4 h) (3). LDL cholesterol was calculated as the difference between cholesterol in the $d > 1.006$ g/mL fraction and HDL cholesterol. The presence of β -VLDL in the $d < 1.006$ g/mL fraction was determined by agarose gel electrophoresis (Cholesterol Profile-15 kit; Helena Laboratories, Beaumont, TX). ApoE phenotype was determined by isoelectric focusing gel electrophoresis followed by immunoblotting of whole serum (14) or by a restriction isotyping method (15). ApoB, apoC-III, and apoE were measured in serum by nephelometry and were measured in RLP fractions by enzyme-linked immunosorbent assay (ELISA) (16, 17).

TABLE 1. Characteristics of hypertriglyceridemic patients

	Type IIb (n = 15)	Type III (n = 15)	Type IV (n = 15)	Significance (IIb vs. IV)
Age (years)	50.4 \pm 3.0	50.8 \pm 2.6	50.5 \pm 2.4	—
BMI (kg/m ²)	30.3 \pm 1.5	30.9 \pm 1.1	29.4 \pm 0.8	—
Total triglyceride	3.7 \pm 0.2 (331.7 \pm 14.8)	3.8 \pm 0.2 (333.6 \pm 18.3)	3.9 \pm 0.30 (344.4 \pm 27.0)	—
Total cholesterol	8.2 \pm 0.3 (316.4 \pm 12.8) ^b	6.6 \pm 0.4 (254.7 \pm 15.4)	5.5 \pm 0.20 (212.0 \pm 7.8) ^a	<i>P</i> < 0.001
VLDL-C	1.6 \pm 0.1 (60.0 \pm 4.5) ^c	2.7 \pm 0.2 (106.1 \pm 8.8)	1.5 \pm 0.1 (58.1 \pm 5.3) ^c	—
LDL-C	5.7 \pm 0.3 (220.3 \pm 11.8) ^c	2.9 \pm 0.2 (111.7 \pm 8.1)	3.0 \pm 0.1 (116.4 \pm 4.1)	<i>P</i> < 0.001
HDL-C	1.0 \pm 0.1 (36.4 \pm 2.2)	1.0 \pm 0.1 (37.0 \pm 1.4)	1.0 \pm 0.1 (37.4 \pm 4.5)	—
ApoB	3.1 \pm 0.1 (168.9 \pm 7.9) ^c	1.5 \pm 0.1 (82.7 \pm 3.7)	2.0 \pm 0.1 (111.7 \pm 2.8) ^c	<i>P</i> < 0.001
ApoC-III	24.4 \pm 1.0 (21.3 \pm 0.9) ^a	20.8 \pm 1.2 (18.2 \pm 1.0)	24.9 \pm 1.8 (21.8 \pm 1.6)	—
ApoE	2.0 \pm 0.1 (6.7 \pm 0.5) ^c	3.6 \pm 0.2 (12.1 \pm 0.6)	1.5 \pm 0.1 (5.1 \pm 0.2) ^c	<i>P</i> < 0.001

Values represent means \pm SE. Plasma lipid levels are expressed in mmol/L and mg/dL (in parentheses); plasma apolipoproteins are expressed in μ mol/L and in mg/dL (in parentheses).

Significantly different from type III patients by unpaired *t*-test or Mann-Whitney rank sum test when group data were not normally distributed: ^a*P* < 0.05, ^b*P* < 0.01, ^c*P* < 0.001.

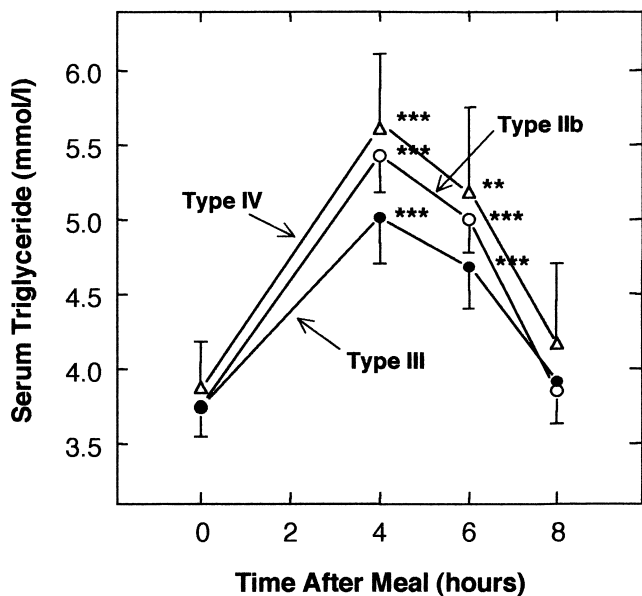


Fig. 1. Mean serum triglyceride concentration of hypertriglyceridemic patients in the fasted (0 h) and fed state (4, 6, and 8 h). Data points represent means \pm SE for type III HLP patients (closed circles), type IIb patients (open circles), and type IV patients (open triangles) ($n = 15$ in each group). Serum triglyceride concentrations were significantly higher in the fed (4 and 6 h) compared with the fasted state in all three patient groups: ** $P < 0.01$, *** $P < 0.001$ by Student's paired t -test. No significant differences, however, were found between mean triglyceride levels (fasted or fed) of different groups.

Statistical analysis

Statistical analyses were performed with Sigmapstat statistical software (Jandel, San Rafael, CA). Student's unpaired t -test was used for determining the statistical significance of mean differences between groups. Mann-Whitney rank sum tests were used

when data sets were not normally distributed. Kolmogorov-Smirnov tests were used to test normality and equal variance of data sets. Paired t -tests were used to compare data at different times during the meal.

RESULTS

The characteristics of the three HTG patient groups are shown in **Table 1**. There were 9 males and 6 females in the type IIb group, 10 males and 5 females in the type III group, and 11 males and 4 females in the type IV group. The apoE phenotype distribution (apoE 4/3, 3/3, 3/2, 4/2) of patients in the type IIb group was $n = 5, 6, 3,$ and 1, respectively, and in the type IV group was $n = 8, 5, 1,$ and 1, respectively. According to selection, all patients in the type III group had an apoE 2/2 phenotype. Also by selection, mean age, body mass index (BMI), and total fasting serum TG concentrations of the three study groups were similar (**Table 1**). Mean HDL cholesterol concentrations were also similar. Type IIb patients had significantly higher serum levels of LDL cholesterol, and hence significantly elevated total serum cholesterol and apoB concentrations. The VLDL fraction in type III patients was characterized by elevated levels of VLDL cholesterol, and these patients had increased levels of serum apoE compared with other patients (**Table 1**).

Ingestion of the fat-rich meal resulted in a significant increase ($P < 0.01$) in serum triglyceride concentration (30–50%) in all patients (**Fig. 1**). Postprandial increase in serum triglyceride tended to be somewhat less in type III patients, although no significant difference was found between mean postprandial triglyceride levels of different groups at 4, 6, or 8 h after the meal. In all three patient groups, serum TG levels in the fed state were significantly

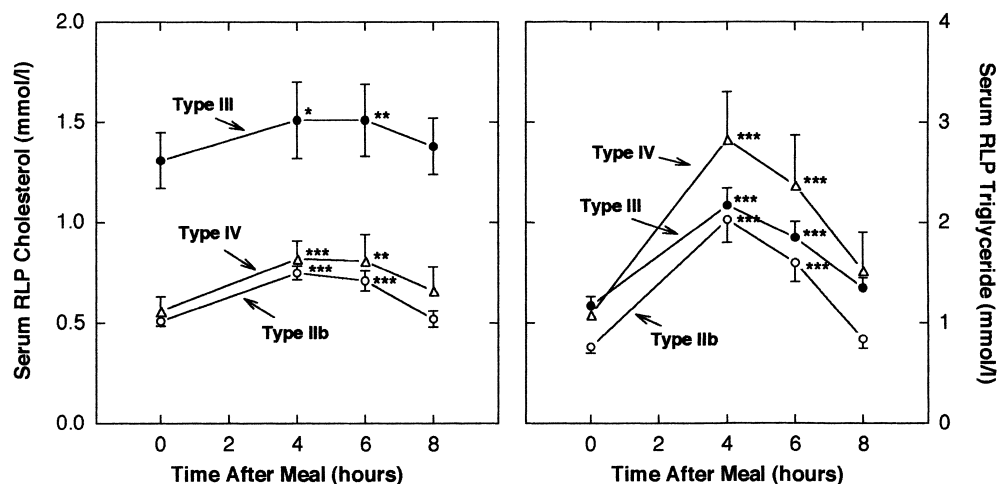


Fig. 2. Effect of fat-rich meal on serum RLP cholesterol and triglyceride concentrations. Data points represent means \pm SE for type III HLP patients (closed circles), type IIb patients (open circles), and type IV patients (open triangles) ($n = 15$ in each group). RLP cholesterol and triglyceride concentrations were significantly elevated in the fed state compared with the fasted state in all three patient groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by Student's paired t -test. Serum RLP cholesterol levels of type III patients were significantly different from those of type IIb and IV patients at all time points ($P < 0.001$). Only the 0- and 8-h RLP triglyceride levels of type IIb patients were significantly different from those of type III patients ($P < 0.01$).

correlated with their level in the fasted state; correlation coefficients (r) between 0 and 4 h TG levels were 0.73 ($P < 0.01$), 0.81 ($P < 0.001$), and 0.78 ($P < 0.001$), and between 0 and 6 h TG levels were 0.67 ($P < 0.01$), 0.76 ($P < 0.01$), and 0.80 ($P < 0.001$) for type IIb, III, and IV patients, respectively.

Mean serum RLP-C and RLP-TG levels in the fasted (0 h) and fed state (4, 6, and 8 h) are shown for the three patient groups in **Fig. 2**. In the fasted state, type III patients had significantly higher RLP-C levels (1.31 ± 0.14 vs. 0.51 ± 0.03 and 0.56 ± 0.07 mm; type III vs. IIb and IV, respectively, $P < 0.001$). They also had significantly higher RLP-TG levels ($P < 0.001$) compared with type IIb patients, but not compared with type IV patients (1.17 ± 0.09 vs. 0.76 ± 0.06 and 1.08 ± 0.15 mm, type III vs. IIb and IV, respectively). Fasting RLP-C levels were significantly correlated with fasting TG levels: $r = 0.55$ ($P < 0.05$), 0.58 ($P < 0.05$), and 0.84 ($P < 0.001$) for type IIb, III, and IV patients, respectively. Fasting RLP-TG levels were also significantly correlated with fasting TG levels: $r = 0.68$ ($P < 0.01$), 0.83 ($P < 0.001$), and 0.90 ($P < 0.001$) for type IIb, III, and IV patients, respectively. Ingestion of the fat-rich meal caused RLP-C and RLP-TG concentrations to increase, and mean levels at 4 and 6 h after the meal were significantly higher than fasting levels in all groups ($P < 0.05$). RLP-C, but not RLP-TG concentrations, were significantly higher in type III patients in the fed state (**Fig. 2**). Postprandial RLP-C levels were significantly correlated with fasting RLP-C, and postprandial RLP-TG levels were significantly correlated with fasting RLP-TG. For example, correlations between fasting and 4-h RLP-C levels were $r = 0.72$ ($P < 0.01$), $r = 0.88$ ($P < 0.001$), and $r = 0.82$ ($P < 0.01$) for type IIb, III, and IV patients, respectively, and between fasting and 4-h RLP-TG levels were $r = 0.61$ ($P < 0.05$), $r = 0.53$ ($P < 0.05$), and $r = 0.70$ ($P < 0.01$). Postprandial increase in levels of serum triglyceride, RLP-C, and RLP-TG were calculated by subtracting fasting concentrations from those at 4, 6, and 8 h after the meal. Mean data for the three groups are compared in **Fig. 3**. Type III patients tended to have smaller and type IV patients tended to have greater postprandial increases in all three parameters; however, no significant differences were found between mean values at any postprandial time point. By 8 h, all three parameters had returned to fasting levels in type IIb and type III patients, although not as completely in type IV patients. Postprandial increase in the concentration of RLP-C was not significantly correlated with fasting serum TG levels (i.e., for data at 4 h, $r = 0.01$ [$P = 0.96$, not significant], 0.39 [$P = 0.17$, NS], and 0.29 [$P = 0.30$, NS] for type IIb, III, and IV patients, respectively). Postprandial increase in the concentration of RLP-C was also not significantly correlated with fasting RLP-C levels (i.e., for data at 4 h, $r = 0.13$ [$P = 0.64$, NS], 0.22 [$P = 0.46$, NS], and 0.19 [$P = 0.50$, NS] for type IIb, III, and IV patients, respectively). Postprandial increases in RLP-TG at 4 and 6 h were also not correlated with fasting TG or fasting RLP-TG concentrations. There was, however, a strong correlation between postprandial increases in RLP-C and postprandial in-

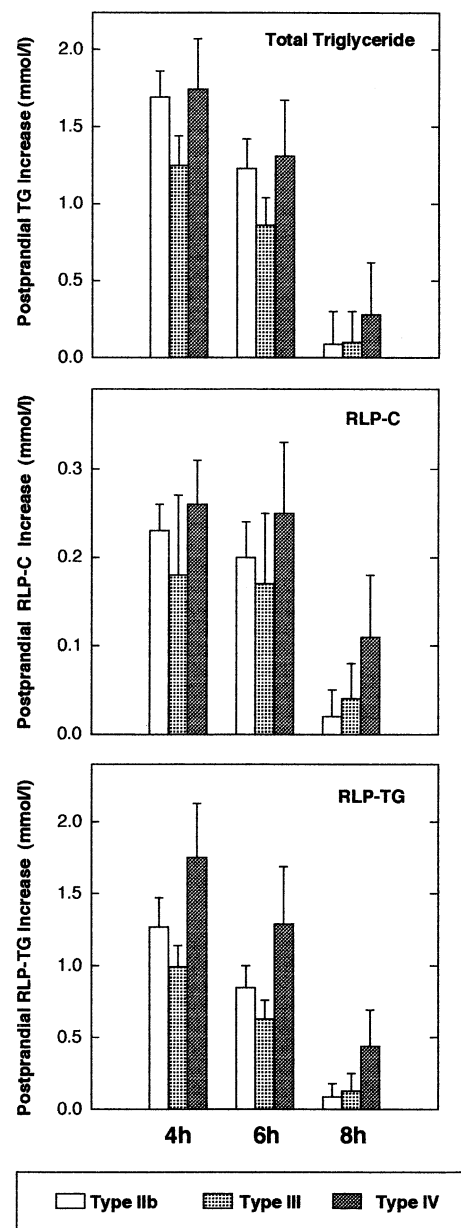


Fig. 3. Postprandial increase in serum triglyceride, RLP cholesterol, and RLP triglyceride concentration of type IIb patients (open bars), type III patients (lightly shaded bars), and type IV patients (heavily shaded bars). No statistically significant differences were found between groups at any time point, although mean postprandial changes tended to be less in type III patients

creases in total TG levels (i.e., for data at 4 h, $r = 0.88$ [$P < 0.001$], 0.67 [$P < 0.05$], and 0.87 [$P < 0.001$]; for data at 6 h, $r = 0.89$ [$P < 0.001$], 0.66 [$P < 0.05$], and 0.93 [$P < 0.001$] for type IIb, III, and IV patients, respectively). Similarly strong correlations existed between postprandial increases in RLP-TG and postprandial increases in total TG levels (i.e., for data at 4 h, $r = 0.82$ [$P < 0.001$], 0.93 [$P < 0.001$], and 0.95 [$P < 0.001$]; for data at 6 h, $r = 0.95$ [$P < 0.001$], 0.92 [$P < 0.001$], and 0.96 [$P < 0.001$] for type IIb, III, and IV patients, respectively).

A striking feature of the data in **Fig. 3** was the magni-

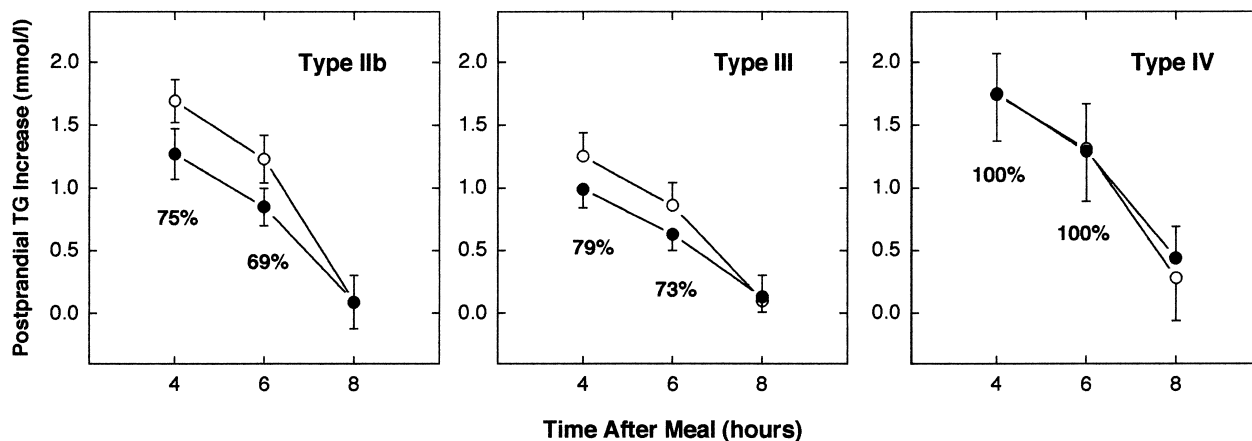


Fig. 4. Comparison between postprandial increase in total serum and RLP triglyceride levels for different patient groups. Mean data (\pm SEM) are shown for total serum (open circles) and for RLP (closed circles). Percentages represent the mean RLP triglyceride increase relative to that of total triglyceride.

tude of the postprandial change in RLP-TG relative to the other two parameters. As shown in **Fig. 4**, more than two-thirds of the postprandial increase in serum TG of type IIb and III patients was accounted for by an increase in RLP-TG, while in type IV patients essentially all of the postprandial increase in serum TG was accounted for by an increase in RLP-TG. Furthermore, RLP-TG increased to a greater extent after the meal than RLP-C, resulting in a significant decrease ($P < 0.01$) in the ratio of RLP-C to RLP-TG in all patients (**Fig. 5**). The RLP fraction was, however, enriched in cholesterol in type III compared

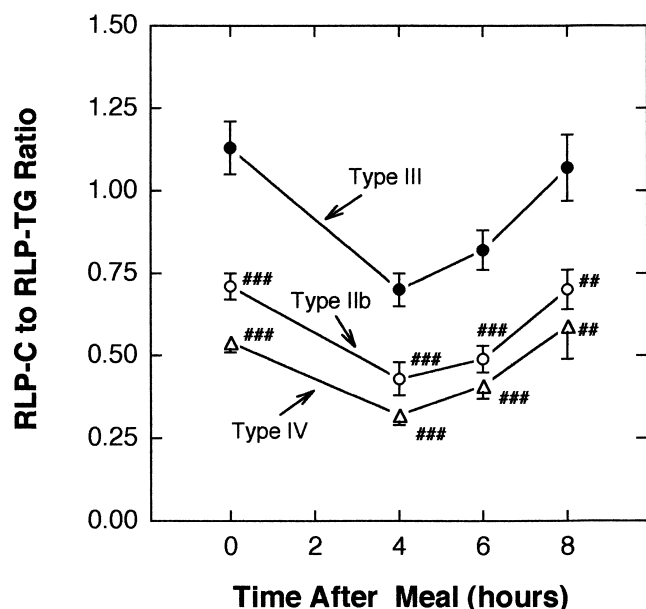


Fig. 5. Cholesterol-to-triglyceride ratio in the RLP fraction of hypertriglyceridemic patients in the fasted (0 h) and fed state (4, 6, and 8 h). Ratios or RLP-C to RLP-TG were significantly lower in the fed state (4 and 6 h) compared with the fasted state (0 h) in all three patient groups ($P < 0.05$ by paired *t*-test). Mean values that were significantly different from those in type III patients are indicated by hatched symbols: ## $P < 0.01$, ### $P < 0.001$.

with other patients in both the fasted and fed state, as reflected by a significantly higher ($P < 0.01$) ratio of RLP-C to RLP-TG at all time points (**Fig. 5**).

Elevated RLP-C concentration and enrichment of the RLP fraction with cholesterol in the fasted state are characteristic features of patients with type III HLP (11–13). The ratio of RLP-C to total serum or plasma TG in the fasted state has thus been used to distinguish type III patients from those with other forms of HTG (13). An RLP-C/TG value of 0.23 has been determined to be a valid cutoff for discriminating type III individuals. As shown in **Fig. 6**, this value was exceeded by 13 of the 15 type III patients in the fasted state (0 h), and by none of the type IIb or IV patients. To determine if this ratio also discriminated type III patients in the fed state, ratios of RLP-C to serum TG were determined for individual patients at 4 and 6 h after the meal. Ingestion of the fat-rich meal did not significantly affect the ratio of RLP-C to TG in type IIb patients (0.138 ± 0.007 , 0.138 ± 0.006 , and 0.140 ± 0.006 ; 0, 4, and 6 h, respectively), or those of type IV patients (0.141 ± 0.011 , 0.144 ± 0.008 , and 0.149 ± 0.011 ; 0, 4, and 6 h, respectively). In contrast, mean ratios did decrease slightly in type III patients and this difference was statistically significant: 0.348 ± 0.030 , 0.301 ± 0.0029 ($P < 0.001$), and 0.320 ± 0.029 ($P < 0.05$); 0, 4, and 6 h, respectively. Despite this decrease, however, 10 of 15 at 4 h and 12 of 15 type III patients at 6 h had a ratio greater than 0.23 in the fed state (**Fig. 6**).

RLP apolipoprotein levels were determined in a subgroup of patients ($n = 6$ for each group) and mean data are shown in **Fig. 7**. The most apparent difference between the type III patients and the others was the significantly increased level of RLP-apoE among the former patients, which were elevated two- to five-fold in both the fasted and fed state. RLP-apoE levels did not increase after the meal in type III patients, but were significantly elevated above fasting levels in type IIb and IV patients at both 4 and 6 h ($P < 0.05$). RLP-apoC-III levels were also significantly higher in the fed state, this being statistically

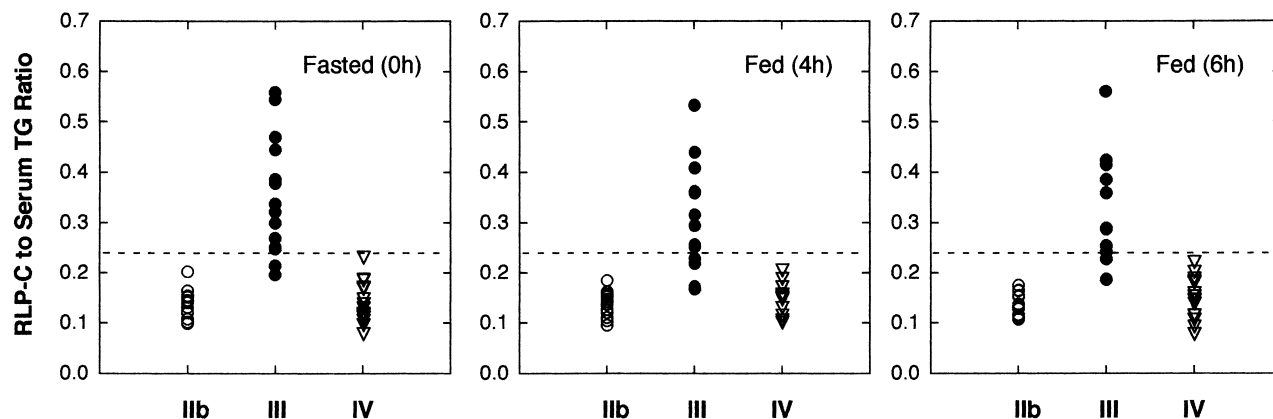


Fig. 6. RLP cholesterol-to-total serum triglyceride ratio in individual patients in the fasted (0 h) and fed state (4 and 6 h). The dashed lines indicate the cutoff value of 0.23, which has been used (ref. 13) for fasted plasma to identify patients with type III HLP.

significant in type IIb and IV patients at 4 h ($P < 0.05$). No significant differences in fasted or fed RLP-apoC-III levels were found between groups. RLP-apoB levels were not significantly altered by the meal, and tended to be higher in type III patients in both the fasted and fed state.

DISCUSSION

The present study has shown that serum RLP-C and RLP-TG levels increase in HTG patients after the ingestion of a fat-rich meal. The magnitude of increase in RLP-C and RLP-TG was similar in type IIb, III, and IV patients, who, by selection, had similar levels of fasting TG. Thus, despite the fact that type III patients had significantly elevated remnant lipoprotein levels in the fasted state (evidenced by the presence of β -VLDL) and also significantly

elevated fasting levels of RLP-C compared with type IIb and IV patients, they did not have an exaggerated postprandial increase in RLP levels. Similarly, type IIb patients had significantly elevated levels of circulating LDL in both the fasted and fed state, yet their postprandial RLP response was similar to that of other patients. Therefore, even though the etiology and molecular mechanisms responsible for hyperlipidemia in these HTG patients were different, they had similar mean fasting TG levels, a similar magnitude of postprandial triglyceridemia, and similar mean postprandial increases in RLP lipids.

These results are consistent with the concept that one of the principal determinants of postprandial triglyceridemia in HTG patients (irrespective of the molecular basis of their HTG) is the fasting triglyceride and/or TRL-TG level. This is because chylomicrons of intestinal origin and VLDL of hepatic origin are catabolized via the same meta-

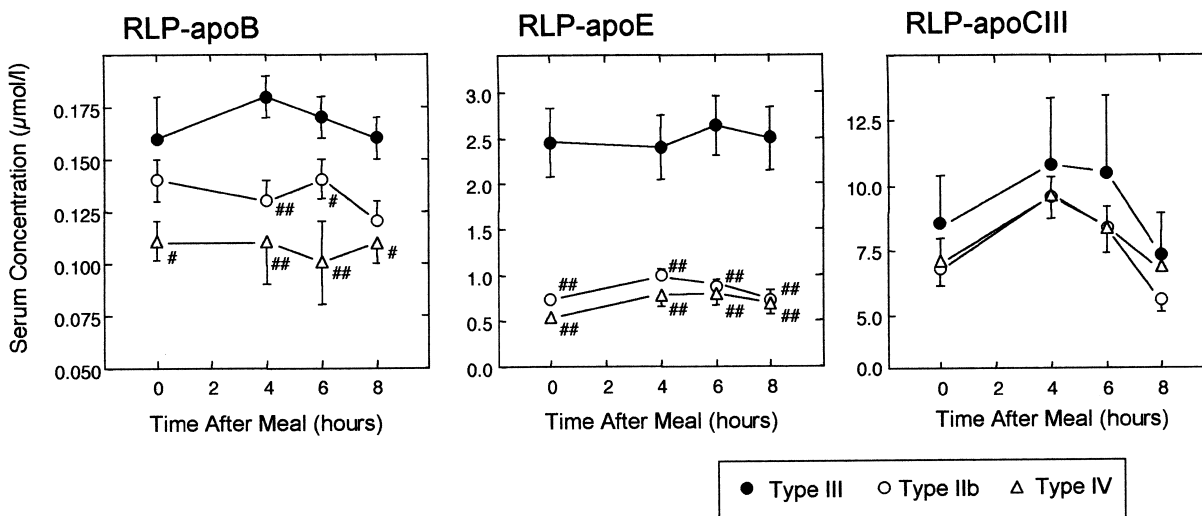


Fig. 7. Serum RLP apolipoprotein concentrations of hypertriglyceridemic patients in the fasted (0 h) and fed state (4, 6, and 8 h). Data points represent means \pm SE for type III HLP patients (closed circles), type IIb patients (open circles), and type IV patients (open triangles) ($n = 6$ in each group). Mean values that were significantly different from those of type III patients are indicated with hatched symbols: # $P < 0.05$, ## $P < 0.01$. A statistically significant increase in RLP-apoE levels was observed in type IIb and IV patients at 4 and 6 h ($P < 0.05$), but not in type III patients. RLP-apoC-III levels were also significantly elevated at 4 h in type IIb and IV patients, but not in type III patients. No significant differences were observed between fasted and fed RLP-apoB levels.

bolic pathway (18–20). Chylomicrons secreted into the blood circulation after the ingestion of a fat-rich meal are catabolized by lipoprotein lipase, with apoC-II as cofactor, the same molecules that are responsible for the hydrolysis of VLDL in the fasted state. The more the lipolytic pathway is saturated in the fasted state, the greater the magnitude of postprandial triglyceridemia. Postprandial triglyceride concentrations are thus significantly correlated with fasting triglyceride levels, and as we have found in the present study, postprandial RLP concentrations are significantly correlated with fasting RLP levels.

Surprisingly, however, we have found that patients with type III HLP did not accumulate postprandial RLP cholesterol or RLP triglyceride to a greater extent than the other HTG patients. Magnitude of postprandial lipemia of type III and type IV patients has been compared previously by Weintraub et al. (21), who showed that postprandial triglyceridemia was not particularly exaggerated in type III patients; however, nonchylomicron “remnant” retinyl ester clearance was noticeably delayed in type III versus type IV patients. Brenninkmeijer et al. (22) have similarly shown that the postprandial increase in plasma TG was no greater in normolipidemic homozygous apoE2 subjects compared with normolipidemic heterozygous or non-apoE2 subjects, although retinyl ester in chylomicrons and chylomicron remnants of homozygous E2 subjects was significantly increased. The present results have also demonstrated that postprandial triglyceridemia of type III patients was not increased compared with that of other HTG patients (Figs. 1 and 3). Because RLP-TG and RLP-C levels were strongly correlated with total plasma TG levels in both the fasted and fed state, the postprandial increase in RLP lipids was also found not to be greater in type III patients. This reflects the fact that in all patients a large proportion of the postprandial increase in total plasma TG was accounted for by an increase in RLP-TG (Fig. 4). Nearly all chylomicrons and chylomicron remnants of intestinal origin therefore appear to have been isolated within the RLP fraction. This raises the question of what should be defined, and what should not be defined as a remnant. Originally, the term “remnant” was applied by Redgrave (23) to those lipoprotein particles that were formed from chylomicrons injected intravenously into functionally eviscerated rats. “Remnant” particles present in the circulation 90 min after injection of chylomicrons had a median diameter of 90 nm compared with a diameter of 200 nm for chylomicrons, and a triglyceride-to-cholesteryl ester mass ratio of 6.1 compared with 64.9 for chylomicrons. It was estimated that 90% of the triglyceride in chylomicrons had to be removed, in order to obtain chylomicron remnants. The concept of a triglyceride-rich lipoprotein remnant was broadened by the *in vivo* kinetic work of Packard et al. (24), who showed that in humans, less than 10% of large VLDL (VLDL₁) apoB was converted to LDL apoB. A significant proportion of VLDL₁ was found to be delipidated to a particle in the small VLDL or IDL density range, and this remnant particle was removed directly from the circulation. Physiologically, remnants have therefore become recognized as partially delipidated

products of TRL (either chylomicrons or VLDL), which are removed rapidly from the circulation by receptor-mediated processes and are not converted to smaller, lower density particles. Methodologically, however, remnant lipoproteins are defined by the biochemical criteria by which they are isolated, for example, intermediate density lipoproteins are density-isolated remnants, β -VLDL are density- and charge-isolated remnants. Remnants isolated by immunoaffinity gel are accordingly remnant-like particles (RLP), defined by their apolipoprotein composition and their immunoaffinity to a specific anti-apoB-100 antibody. The present results demonstrate that in the fed state this fraction contains a significant quantity of both large as well as small chylomicrons. These lipoproteins contain apoB-48 and not apoB-100 and are therefore not bound by the immunoaffinity gel. Some would suggest that this makes the RLP fraction less physiologically “remnant-like”; however, others would argue that any chylomicron in the venous system has undergone some lipolytic modification (as well as apolipoprotein modification, e.g., loss of apoA-I) and should therefore be considered a remnant. In actual fact, no methodologically defined remnant fraction completely and perfectly encompasses our current physiologic concept of a TRL remnant. Different approaches to isolating TRL remnants have nevertheless taught us a great deal about remnant metabolism and the contribution of these lipoproteins to the pathogenesis of atherosclerosis (1).

Although the present study represents the first investigation of RLP levels in well-defined HTG patients in the fed state, three previous studies have measured RLP levels after the ingestion of an oral fat load (25–27). The results of these studies are summarized in **Table 2**, together with the results of the present study. Although a normolipidemic control group was not included in the present study, and comparing data from different laboratories is not ideal, it is apparent that HTG patients tend to have greater postprandial increases in RLP-C and RLP-TG levels relative to healthy controls. In addition, it can be seen that the postprandial increase in RLP levels tends to be greater in individuals with angiographically defined CAD (25) and in CAD patients with restenosis (26), lending support to the concept that postprandial lipoproteins and specifically postprandial TRL remnants may play an important role in the onset and development of CAD (28, 29).

In postprandial studies, there has been considerable interest in defining the contribution of intestinal and hepatic TRL to postprandial hypertriglyceridemia (30). It is now generally accepted that the bulk of postprandial increase in TG is due to the accumulation in plasma of intestinal apoB-48-containing chylomicrons, while the postprandial increase in TRL apoB is largely due to the accumulation in plasma of hepatic apoB-100-containing VLDL (31–33). In terms of particle number, one can therefore speculate that there are a larger number of apoB-100-containing remnants circulating in the fed state compared with apoB-48-containing remnants. We have previously shown that in the RLP fraction isolated from normolipidemic subjects after an overnight fast, virtually all (98%)

TABLE 2. Summary of postprandial RLP studies

Subjects	Meal	Result		Reference
		FTG	ΔPP	
		<i>mmol/L</i>		
Normolipidemic men (n = 15)	Cream (40 g of fat per m ²)	1.09	RLP-C = 0.02 RLP-TG = 0.60	25
Normolipidemic men with CAD (n = 15)		1.08	RLP-C = 0.32 RLP-TG = 1.76	
CAD patients (n = 7)	Oral fat load (unspecified amount of fat)	0.97	RLP-C = 0.06	26
CAD patients with restenosis (n = 10)		1.85	RLP-C = 0.12	
Healthy adults (9 women, 10 men)	Cream (70 g of fat)	0.81	RLP-C = 0.09 RLP-TG = 0.81	27
Hypertriglyceridemic patients (n = 45)	Fat-rich meal (45 g of fat)	Type IIb = 3.7	RLP-C = 0.23 RLP-TG = 1.27	Present study
		Type III = 3.8	RLP-C = 0.18 RLP-TG = 0.99	
		Type IV = 3.9	RLP-C = 0.26 RLP-TG = 1.75	

Abbreviations: CAD, coronary artery disease; ΔPP, maximum postprandial increase; FTG, fasting triglyceride concentration.

of RLP-apoB is apoB-100. In type IV patients, 90% of RLP-apoB was apoB-100, while in type III patients almost half of RLP-apoB was apoB-48 (12). Unfortunately, small sample numbers prevented us from determining ratios of RLP-apoB-48 to apoB-100 in the present study; however, measurement of total RLP apoB levels showed that ingestion of the fat-rich meal did not cause a postprandial increase in total number of RLP particles in any of the patient groups (Fig. 7). RLP-apoC-III levels did, however, increase postprandially in all patients. We speculate that even though there was no postprandial change in total RLP-apoB concentration, there may well have been an increase in RLP-apoB-48 with a concomitant decrease in RLP-apoB-100, most likely due to competition between apoB-100- and apoB-48-containing TRL for lipolysis, as already discussed (18–20).

One of the most apparent features of the present data is the fact that type III patients had clearly increased levels of RLP-C and RLP-apoE in both the fasted and fed state compared with patients with other forms of HTG. This result was expected considering that the etiology of remnant lipoprotein and plasma apoE accumulation in type III patients is well understood [i.e., the primary defect is impaired binding of apoE2 on TRL to hepatic receptors (10)]. RLP-C levels are elevated to such a pronounced extent in the fasted state in type III patients that their ratio of RLP-C to TG can be used to distinguish them from HTG patients with other forms of HLP (13). The present results clearly demonstrate (Fig. 6) that this is also true for type III patients in the fed state, which means that the ratio of RLP-C to TG is a diagnostic tool for type III HLP using either nonfasted or fasted serum samples.

In conclusion, the present results have indicated that: 1) RLP-C and RLP-TG levels are significantly increased in the fed versus fasted state in HTG patients; 2) patients

with different forms of HTG, but similar TG levels, have similar postprandial increases in RLP-C and RLP-TG; and 3) compared with patients with other forms of HTG, type III patients have significantly elevated levels of RLP-C and RLP-apoE in both the fasted and fed state. ■■

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