Determinants of postprandial lipemia in men with coronary artery disease and low levels of HDL cholesterol

M. Syvänne,[1,*] P. J. Talmud,[†] S. E. Humphries,[†] R. M. Fisher,[†] M. Rosseneu,[§] H. Hilden,[*] and M-R. Taskinen[*]

Department of Medicine,* University of Helsinki, Helsinki, Finland; The Centre for Genetics of Cardiovascular Disorders,† University College and Middlesex School of Medicine, Department of Medicine, The Rayne Institute, London, U.K.; and Laboratorium voor Lipoproteine Chemie,§ Fakulteit der Geneeskunde, Universiteit Gent, Gent, Belgium

Abstract We studied the determinants of postprandial lipemia in 49 post-coronary-bypass men with low HDL cholesterol (≤1.1 mmol/l at screening). The subjects were given a mixed meal containing 63 g fat and 150,000 IU vitamin A. Serum was obtained before and 3, 4, 5, 6, and 8 h after the meal. $S_f > 400$ and S_f 12–400 lipoproteins, LDL, and HDL were separated by ultracentrifugation; and triglyceride (TG), retinyl ester (RE), and apolipoprotein (apo)E concentrations were measured. The associations of 15 potential predictor variables with measures of postprandial lipemia were evaluated in univariate and multivariate models. Fasting TG concentration was the most important determinant of postprandial lipid and apoE concentrations. In univariate analyses, neither apoE phenotype nor common genetic polymorphisms in the apoB gene (*Xba* I and apoB signal peptide length polymorphisms), lipoprotein lipase gene (*Hind* III polymorphism), or apoC-III gene (C_{1100} to T sequence change) significantly predicted the magnitude of postprandial lipemia. In multivariate linear regression analyses, fasting TG concentration ($P < 0.001$) and postheparin plasma hepatic lipase activity ($P = 0.023$) were directly, and body mass index ($P = 0.007$) and the presence of apoE2 ($P = 0.029$) allele inversely related to the TG increment in $S_f > 400$ lipoproteins. Fasting TG was associated with a high ($P < 0.001$) and presence of the SP24 allele of the apoB signal peptide gene with a low ($P = 0.014$) S_f 12–400 TG response. Fasting TG concentrations alone predicted 35%, 10%, and 34% of the variability in postprandial $S_f > 400$ responses of TG, RE, and apoE; multivariate models improved this predictive power to 40–50%. Even multivariate models were poor predictors of postprandial responses in S_f 12–400 lipoproteins (0–26%). Much of the interindividual variation in the magnitude of postprandial lipemia remained unexplained in the present study.—**Syvanne, M., P. J. Talmud, S. E. Humphries, R. M. Fisher, M. Rosseneu, H. Hilden, and M-R. Taskinen.** Determinants of postprandial lipemia in men with coronary artery disease and low levels of HDL cholesterol. *J. Lipid Res.* 1997. **38:** 1463–1472.

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Several studies in recent years have identified the magnitude of postprandial lipemia as a risk marker of the presence (1, 2) or progression (3) of coronary artery disease. Therefore, factors that regulate lipoprotein metabolism in the postprandial state are of interest to preventive cardiology.

It is well established that fasting serum triglyceride (TG) concentrations show a positive correlation with the degree of postprandial lipemia. Fasting hypertriglyceridemia itself is a risk factor, albeit a debated one, of atherosclerosis (4). However, TG concentrations in the postprandial state, which stresses the fat-transporting and fat-metabolizing capacity of the organism, have been found to be a more sensitive marker of coronary disease than fasting values (2). The correlation coefficient between fasting and incremental postprandial TG concentrations varies from 0.4 to 0.6 (5–7). Although this correlation is strong and highly statistically significant, differences in fasting TG levels only partially account for the interindividual variation in the magnitude of postprandial lipemia.

The regulation of exogenous lipid metabolism can potentially occur at many stages. Ingested lipid is digested, absorbed into enterocytes, resynthesized, and packaged into chylomicron (CM) particles, which are secreted via lymphatic vessels into the bloodstream where they acquire several additional apolipoproteins

Abbreviations: TG, triglyceride; CM, chylomicron; LPL, lipoprotein lipase; apo, apolipoprotein; CETP, cholesteryl ester transfer protein; BMI, body mass index; RE, retinyl ester; PCR, polymerase chain reaction; HL, hepatic lipase.

[1]To whom correspondence should be addressed.

(8). In peripheral capillaries, TGs are hydrolyzed by lipoprotein lipase (LPL), requiring apolipoprotein (apo)C-II as a cofactor (9). The resulting CM remnant particle is removed from the plasma compartment via hepatic receptors (10).

The intestinally derived lipoproteins are in constant interaction with other lipoprotein classes. They compete with the liver-derived very low density lipoprotein (VLDL) particles for access to LPL and possibly for removal sites in the liver. They exchange surface and core constituents with other lipoproteins, especially high density lipoprotein (HDL). ApoE, which is necessary for the interaction between CM remnants and hepatic receptors (11), is transferred from HDL. HDL is further an acceptor for surface material that becomes redundant after TG hydrolysis, notably the apoC peptides which otherwise inhibit the interaction of the remnant particles with hepatic receptors (12). Core lipids are transported between lipoprotein classes by the cholesteryl ester transfer protein (CETP); TG is transferred from the CMs and VLDL to HDL, and cholesteryl esters are reciprocally transferred from HDL to the TG-rich lipoproteins (13).

Thus, a range of apolipoproteins, enzymes, cofactors, and transport proteins are involved; each are under genetic control and interact with the lipoproteins in the postprandial state, potentially modifying the magnitude of postprandial lipemia.

A low HDL cholesterol concentration is a well-known risk factor for coronary atherosclerosis (14) and is also associated with a high postprandial level of lipemia (15). We are currently involved in a prospective trial addressing the question of whether gemfibrozil, a lipid-modifying agent of the fibrate group, prevents the progression of coronary artery disease as assessed by quantitative coronary angiography in post-coronary-bypass men whose main lipid abnormality is low HDL cholesterol (16). In a subgroup of these men, we performed oral fat-load tests at baseline and during the double-blind treatment period with gemfibrozil or placebo. The main purpose of this substudy is to determine whether postprandial lipemia predicts angiographic progression of coronary atherosclerosis; these results will be reported later when available. The present report deals with the determinants of postprandial lipemia in a cross-sectional setting at the baseline of the study. The rationale for this evaluation is that we have a well-defined group of patients in whom extensive studies of metabolic variables potentially important as determinants of postprandial lipemia have been performed. We evaluated the impact of age, adiposity, fasting serum TG, the endothelial TG hydrolases, CETP, glucose intolerance, and insulin concentration. We also studied the impact of several potentially relevant genetic polymorphisms on the magnitude of postprandial lipemia.

SUBJECTS AND METHODS

Subjects

Fifty nonsmoking men were randomly selected for studies on postprandial lipid metabolism among 203 participants in a randomized trial. These 203 men constitute the study population at Helsinki University Central Hospital in a three-center study, the other participating centers being Oulu and Tampere University Hospitals. This study, the Lopid Coronary Angiography Trial (LOCAT), has enrolled a total of 395 men who have previously undergone coronary bypass surgery. In addition to male sex and a former bypass operation, the main entry criteria into this study were screening HDL cholesterol ≤1.1 mmol/l (by precipitation), serum TG ≤4.0 mmol/l, and calculated LDL cholesterol ≤4.5 mmol/l. Exclusion criteria included manifest diabetes, body mass index (BMI) >30 kg/m^2 at screening, use of lipid-lowering medications, and renal, hepatic, or thyroid dysfunction likely to affect lipoprotein metabolism (16). None of the patients were taking diuretics, angiotensin-converting enzyme inhibitors, or calcium channel blockers, but 43 subjects received β-blockers (metoprolol, 31; atenolol, 7; pindolol, 2; sotalol, 2; and bisoprolol, 1 subject). All subjects received dietary counseling during the screening phase of the study, prior to the metabolic studies, and were advised to consume a diet containing 30% of energy as fat, with saturated, monounsaturated, and polyunsaturated fat each contributing 10%. All patients signed an informed consent to participate in the study. The protocol was approved by the Ethics Committees of the participating hospitals.

Measurements

Oral fat-load test. Blood samples were taken from an indwelling catheter in a forearm vein at 7:30 AM after an overnight fast. The subjects then consumed a fat-rich mixed meal consisting of bread, butter, cheese, sliced sausage, a boiled egg, fresh paprika, soured whole milk, orange juice, and coffee or tea. The meal was eaten within 15 min under observation. Half-way through the meal 150,000 units of Vitamin A (retinyl palmitate in soya oil, Leiras, Turku, Finland) was taken to label the intestinally derived particles with retinyl esters (RE). Although we recognize the limitations of RE as specific markers of CM remnants (17), the transfer of RE from CMs to other lipoprotein classes during an 8-h fat-load test is limited (18); therefore RE levels serve as useful estimates of the intestinally derived component of postprandial lipemia (19). The meal contained 840 kcal energy of which 68.5% came from fat, 17.0% from protein, and 14.5% from carbohydrates. The absolute amount of fat in the meal was 63 g, with a polyunsaturated to

saturated ratio of 0.08. The cholesterol content of the meal was 490 mg. Apart from a feeling of oversatiety and occasional mild headache, the test was tolerated well by the subjects. There was a question of compliance in one subject, and he was excluded from the data analyses. Postprandial blood samples were taken 3, 4, 5, 6, and 8 h after the meal, during which time only water was allowed. Serum was promptly separated in a chilled centrifuge (3000 rpm, 10 min) and protected from light at all stages of processing.

Separation of lipoprotein fractions. The serum sample (3 ml) was placed in Beckman Ultra Clear 14 × 95 mm (13 ml) tubes (Beckman Inc., Palo Alto, CA) and carefully overlayered with 2.5 ml of d 1.006 g/ml sodium bromide. The CM fraction (S_f >400) was isolated by ultracentrifugation for 30 min at 18,000 rpm and at 10°C in a Beckman L8-70 ultracentrifuge with a SW 40 Ti rotor. The CMs were recovered by aspiration of 2 ml from the top of the tube. CM-free serum (2 ml) was placed in Beckman 13 × 51 mm (3 ml) tubes with 1 ml d 1.019 g/ml NaBr and spun for 3 h at 100,000 rpm and at 20°C in a Beckman Optima TL (table-top) ultracentrifuge with a TLA-100.3 rotor. The top fraction containing the S_f 12–400 lipoproteins was harvested. The bottom fraction (2 ml) was placed in Beckman 3-ml tubes and overlayered with 0.3 ml d 1.535 g/ml NaBr and 0.7 ml d 1.060 g/ml NaBr. Centrifugation in the table-top ultracentrifuge was carried out for 2 h at 100,000 rpm and at 20°C, and the LDL fraction was recovered from the top of the tubes. The bottom fraction, containing the HDL and non-lipoprotein serum, is denoted HDL. In preliminary experiments, the LDL fraction was found to contain no or minimal amounts of apoA-I, and the HDL fraction was likewise virtually free of apoB.

The TG recoveries in the fractions ranged from 91 ± 4% to 95 ± 5% (mean ± SD) of unfractionated serum TG at the various sampling times.

DNA preparation and analysis. Blood for DNA extraction was collected after an overnight fast, and DNA was extracted by the salting out method (20). Polymerase chain reaction (PCR) in a total volume of 50 µL was performed on a Cambio "intelligent" heating block (Cambio, Cambridge, U.K.). In each PCR, 0.5 unit of *Thermus aquaticus* polymerase (Gibco BRL) and 100 to 200 ng of each primer were used.

DNA polymorphisms. Primer sequences and procedures for PCR amplification and subsequent typing have been described for the *Xba* I polymorphism in exon 26 of the apoB gene (21) and for the apoB signal peptide length polymorphism (22, 23). For the *Hind* III polymorphism between exons 8 and 9 in the LPL gene, primer sequences and procedures for PCR amplification and subsequent typing were as described (20). For the C_{1100} to T sequence change in the apoC-III gene, primer sequences and procedures for PCR amplification, sequences of allele specific oligonucleotide probes, procedures for ^{32}P-labeling, oligomelting, and autoradiography have been described (24).

Laboratory determinations. TG concentrations in unfractionated serum and in the lipoprotein fractions were determined by an enzymatic assay in the automated Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland). The coefficient of variation in serum TG determinations in our laboratory is 2.7%. RE concentrations were measured by high performance liquid chromatography as described by Ruotolo et al. (25). The Beckman Ultrasphere Si 5 µm 4.6 mm × 25 cm (No. 235341) column was used. The mobile phase consisted of 900 ml hexane, 150 ml n-butyl chloride, 50 ml acetonitrile, and 150 µl acetic acid, with a flow rate of 2 ml/min. Retinol acetate (R-4632, Sigma Chemical Co., St. Louis, MO) was added to the samples as an internal standard.

Aliquots of serum and the lipoprotein fractions obtained before and 3, 5, and 8 h after the fatty meal were frozen at −20°C and shipped on dry ice to Brugge, Belgium, where apoE concentration was measured by ELISA as described (26).

An oral glucose tolerance test (75 g glucose) was performed at the time of baseline coronary angiography, usually within a month from the fat-load test. In this report, fasting serum insulin and 2-h post-load glucose concentrations were used. Shortly after the angiogram, at the randomization visit, postheparin plasma LPL and hepatic lipase (HL) activities were determined after an overnight fast as described earlier (27, 28). In addition, postabsorptive serum was obtained for lipoprotein fractionation by sequential ultracentrifugation (16). CETP activity was measured as reported previously (29, 30). ApoE phenotyping was done in serum by the method of Havekes et al. (31). The details of these and other laboratory methods used in this study have been be reported separately (16).

Statistical methods. Postprandial lipemia or apolipoproteinemia was quantified using the time-dependent concentration curves of TG, RE, and apoE during the fat-load test. The total postprandial response was determined by the trapezoid rule (32) as the area under the concentration curve and the zero concentration level. The change in TG and apoE concentrations in the postprandial period was calculated similarly but after subtraction of the fasting level from each subsequent value. For RE, the total response is equal to the change as fasting RE concentrations are virtually zero.

To study the determinants of postprandial lipoprotein metabolism, the following continuous variables were evaluated as potential predictors: age, BMI, fasting TG concentration, HDL cholesterol concentration, postheparin plasma LPL and HL activities, CETP activity, glucose concentration 2 h after ingestion of 75 g of

glucose, and the fasting serum insulin concentration. The univariate associations of each of these variables and selected measures of postprandial lipemia were assessed by Spearman's rank correlations.

The patients were dichotomized with respect to the presence or absence of apoE allele ε2 and ε4, the *Xba* I polymorphism "X+" allele, the apoB signal peptide deletion (SP24) allele, the "H−" allele of the *Hind* III LPL gene polymorphism, and the "T" allele of the apoC-III gene. Measures of postprandial lipemia in these groups were compared by the Mann-Whitney *U* test.

The joint influence of the 9 continuous and 6 categorical (genetic) predictors of postprandial lipemia was evaluated by multivariate linear regression analysis. As an exploratory procedure, automatic stepwise regression (33) was used. The change in postprandial concentration of TG, RE, and apoE in the CM (S_f >400) and in the S_f 12–400 fractions was entered, in turn, as the dependent variable and the 15 candidate predictors as the independent variables. Stepwise regression with alpha-to-enter 0.10 and alpha-to-remove 0.10 was used to select the variables significantly ($P < 0.05$) related to the dependent variable. The "tolerance" option was used to prevent multicollinearity (33). Based on these preliminary analyses, fixed multivariate linear regression models were constructed to study the determinants of TG, RE, and apoE responses in the CM and S_f 12–400 fractions.

RESULTS

Table 1 lists characteristics of the study subjects, fasting lipid and lipoprotein concentrations at the time of the fat-load test, and the data on postload glucose and fasting insulin concentrations. In some instances the lipid and BMI values slightly exceeded the screening limits because of fluctuation over time, although all subjects had had acceptable values at screening. As reported elsewhere (16), the HDL cholesterol values based on ultracentrifugation were slightly higher than those obtained by precipitation with phosphotungstic acid and magnesium chloride; according to the latter method, mean HDL cholesterol concentration in our study was 0.82 mmol/l (16). Although patients with manifest diabetes were excluded at screening, 14 subjects had impaired glucose tolerance defined as 2-h postload blood glucose concentration ≥6.7 mmol/l. Based on a fasting insulin cutoff point of 10.0 mU/l, 7 subjects were hyperinsulinemic. Table 1 also indicates the apoE phenotype distribution; and the distributions of the apoB *Xba* I, apoB signal peptide length, LPL *Hind* III, and apoC-III C_{1100} to T sequence change polymorphisms.

TABLE 1. Characteristics of the study subjects, fasting lipid and lipoprotein concentrations, post-load glucose and fasting insulin concentrations, apolipoprotein E phenotypes, and genetic polymorphisms

Number of subjects	49	
Age, yr	60 ± 6	(38 to 70)
Body mass index, kg/m^2	26.3 ± 2.8	(20.5 to 30.4)
TG, mmol/l	1.68 ± 0.81	(0.51 to 4.66)
Cholesterol, mmol/l	5.02 ± 0.71	(2.87 to 6.47)
LDL-C, mmol/l	3.34 ± 0.54	(1.71 to 4.53)
HDL-C, mmol/l	1.02 ± 0.18	(0.56 to 1.37)
2-h Glucose, mmol/l	6.0 ± 1.8	(2.9 to 11.7)
Insulin, mU/l	7.7 ± 3.8	(2.7 to 23.1)
ApoE phenotype, n		
3/2	5	
4/2	1	
3/3	24	
4/3	18	
4/4	1	
ApoB genotype (*Xba*I), n		
X+X+	9	
X+X−	23	
X−X−	15	
ApoB signal peptide genotype, n		
SP27/SP27	29	
SP27/SP24	12	
SP24/SP24	7	
Lipoprotein lipase genotype (*Hind*III), n		
H+H+	30	
H+H−	17	
H−H−	1	
ApoC-III genotype		
CC	21	
CT	22	
TT	5	

TG indicates triglyceride; C, cholesterol; 2-h glucose, blood glucose concentration 2 h after a 75-g oral glucose load; Insulin, fasting serum insulin concentration; Apo, apolipoprotein; SP27 and SP24, alleles encoding apoB signal peptides containing 24 and 27 amino acids, respectively; and C and T, the C and T alleles, respectively, of the C_{1100} to T sequence change in the apoC-III gene. Data were missing for 2 subjects on apoB genotype; and for 1 subject on apoB signal peptide, lipoprotein lipase, and apoC-III genotypes.

Univariate analyses. **Table 2** shows the univariate correlations between measures of postprandial serum TG, RE, and apoE responses and the continuous predictor variables. Only fasting serum TG concentrations were consistently associated with the measures of postprandial lipemia. In addition, there was a significant negative association between the total TG response and postheparin plasma LPL activity, and a similar albeit nonsignificant trend to association between the TG increment and LPL. LPL was not related to RE or apoE concentrations after the fatty meal. There was a positive relation between the total apoE response and CETP activity.

None of the genetic variants including apoE phenotype significantly influenced the magnitude of postprandial lipemia in univariate analyses (**Table 3**). The

TABLE 2. Spearman rank correlations between measures of postprandial lipemia in whole serum and continuous variables

	TG, Total	TG, Change	RE, Total	ApoE, Total	ApoE, Change
Age	−0.009	0.017	0.040	0.145	−0.256
Body mass index	−0.030	−0.169	−0.260	0.044	−0.153
Fasting serum triglyceride	0.914[c]	0.681[c]	0.374[b]	0.617[c]	0.502[c]
HDL cholesterol	−0.071	−0.063	0.077	0.113	−0.147
LPL	−0.340[a]	−0.311	−0.049	−0.106	−0.272
HL	0.121	0.194	0.250	−0.176	0.065
CETP	−0.092	−0.168	−0.104	0.335[a]	0.014
2-h Postload glucose	−0.046	−0.014	0.136	−0.172	0.112
Fasting serum insulin	0.186	0.020	0.039	−0.040	−0.039

TG indicates trigyceride; Total, total postprandial response (see Methods); Change, postprandial change from fasting level (see Methods); RE, retinyl ester; apo, apolipoprotein; HDL, high density lipoprotein; LPL and HL, postheparin plasma lipoprotein lipase and heparin lipase activity, respectively; and CETP, cholesteryl ester transfer protein actitivy.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

same was true for the lipoprotein fractions (data not shown). Those patients taking β-blockers (n = 43) had measures of postprandial lipemia similar to those not on β-blockers (n = 6, data not shown).

Multivariate analyses. **Table 4** shows the results of the multivariate linear regression analyses. The strongest independent predictor of the incremental response of either TG, RE, or apoE in the CM fraction was fasting TG concentration as indicated by the large standardized regression coefficients. BMI tended to be inversely related to the lipemic changes in the CM fraction. HL activity showed a significant positive relation to CM TG, RE, and apoE responses. In this multivariate analysis, the presence of apoE2 was associated with a smaller lipemic response (TG and RE) but was not related to change in apoE concentrations. The postload 2-h glucose concentration was related to RE but not to TG or apoE levels in the CM fraction. As shown by the adjusted R^2 values in Table 4, these models predicted 40% to 50% of the variation in the dependent variables. Fasting TG concentration alone predicted 35%, 10%, and 34% of postprandial CM TG, RE, and apoE responses, respectively. Thus, although fasting TG was the most important predictor, the multivariate models had greater power to predict the magnitude of postprandial lipemia.

Table 4 also shows that models selected by stepwise linear regression had less power to predict postprandial lipemia in the S_f 12–400 fraction than in the CM fraction. For the incremental TG response in the S_f 12–400 fraction, the fasting TG level was again the strongest predictor. However, the presence of the apoB signal peptide deletion allele (SP24) was negatively related to the S_f 12–400 fraction TG response, i.e., its presence predicted a smaller response compared with its absence. The S_f 12–400 fraction RE response had an inverse relation to the apoC-III C_{1100} to T allele, in line with the nonsignificant univariate trend observed for the serum RE response (Table 3). Postheparin plasma HL activity was another significant predictor of the S_f 12–400 fraction RE response after a fatty meal. Notably, fasting TG values were not related to RE concentrations in the S_f 12–400 lipoproteins.

DISCUSSION

This study demonstrates the importance of fasting serum TG concentration as the primary determinant of the magnitude of postprandial lipemia in men with coronary artery disease and low HDL cholesterol concentrations. Our findings are in agreement with several previous observations in different study populations (e.g., refs. (5–7)). Although our study population was a highly selected one, few exclusions in screening were due to excessively high TG levels (fasting serum TG >4 mmol/l). Therefore, with this reservation, the TG concentration range of our study population is probably representative of coronary patients in general. Ooi, Simo, and Yakichuk (34) showed that men with hypoalphalipoproteinemia and mild hypertriglyceridemia had greatly enhanced postprandial lipemia compared with normolipidemic control subjects. Their criterion of hypertriglyceridemia was a serum TG >2.0 mmol/l, with an average level of 3.8 mmol/l. The present study shows that even when frankly hypertriglyceridemic subjects (serum TG >4.0 mmol/l at screening) are excluded, fasting TG remains the main determinant of

TABLE 3. Postprandial lipemia by genetic polymorphisms

	ApoE2− n = 43	ApoE2+ n = 6	ApoE4− n = 29	ApoE4+ n = 20	X− n = 15	X+ n = 32	SP24− n = 29	SP24+ n = 19	H− n = 18	H+ n = 30	T− n = 21	T+ n = 27
TG, total	19.7 ± 9.2	25.9 ± 11.9	21.3 ± 11.0	19.2 ± 7.2	19.6 ± 9.2	20.7 ± 10.2	19.8 ± 10.2	21.3 ± 9.1	21.2 ± 10.2	19.9 ± 9.5	20.9 ± 11.0	20.0 ± 8.7
TG, change	7.1 ± 4.5	7.4 ± 4.0	7.2 ± 4.3	7.2 ± 4.6	8.1 ± 5.4	6.7 ± 4.0	7.5 ± 5.0	6.6 ± 3.5	7.5 ± 4.2	6.9 ± 4.6	7.0 ± 4.6	7.2 ± 4.4
RE, total	35.2 ± 19.9	25.1 ± 10.3	30.5 ± 15.9	38.9 ± 22.7	33.9 ± 14.3	34.5 ± 21.8	30.3 ± 14.9	40.0 ± 23.9	31.5 ± 19.1	35.7 ± 19.7	38.7 ± 20.4	30.6 ± 18.1
ApoE, total	38.3 ± 9.2	48.0 ± 21.7	42.0 ± 12.8	35.9 ± 8.5	36.2 ± 5.6	40.3 ± 11.4	38.7 ± 9.6	41.5 ± 13.9	42.2 ± 14.6	38.4 ± 9.0	41.3 ± 11.8	38.6 ± 11.3
ApoE, change	−2.6 ± 5.1	3.7 ± 8.5	−1.4 ± 6.3	−2.5 ± 5.3	−2.6 ± 5.1	−2.0 ± 6.0	−2.8 ± 5.2	−0.9 ± 6.4	−0.9 ± 3.9	−2.7 ± 6.6	−2.3 ± 6.7	−1.9 ± 5.0

− Indicates absence; +, presence; apo, apolipoprotein; E2 and E4, ε2 and ε4 allele, respectively, of the apoE gene assessed as the apoE phenotype; X, X+ allele of the *Xba*I polymorphism; D, apoB signal peptide deletion (SP24) allele; H, homozygosity for the LPL *Hind*III polymorphism H+ allele; T, apoC-III C_{1100} to T allele; TG, triglyceride; RE, retinyl ester; total, total postprandial response (see Methods); and change, postprandial change from fasting level (see Methods). Values are mmol/l · h (TG), μmol/l · h (RE), or mg/dl · h (apoE); mean ± SD. No differences significant at $P < 0.05$ (Mann-Whitney U test).

postprandial lipemia in a group of men with a mean serum TG concentration of 1.68 mmol/l.

Postheparin plasma HL activity was positively associated with the TG, RE, and apoE responses in the CM fraction and with the RE response in the S_f 12–400 fraction (Table 4). This relationship may seem unexpected, as HL is believed to have a role as a TG hydrolase of IDL and LDL (9) and possibly in the removal of remnant lipoproteins (35). Thus, if anything, one would expect an inverse relation between postprandial lipemia and HL activity. Indeed, Weintraub, Eisenberg, and Breslow (36) reported an inverse association between non-CM RE response and HL activity in normal subjects, but an absence of any significant relation in hypertriglyceridemic patients. Our observations of a positive relation between HL activity and postprandial lipemia are in line with data by Patsch and coworkers (37) as well as a previous study by some of us (38). One explanation for the positive relation between HL activity and postprandial lipemia might be the presence of a common underlying factor or factors. Indeed, Katzel et al. (39) have shown that in men with silent myocardial ischemia hypertriglyceridemia, abdominal obesity, hyperinsulinemia, and a low HDL_2 concentration coexist with a high HL activity. Secondly, experiments in rabbits have suggested that remnant lipoproteins may induce the synthesis of HL (40). Thus, a high HL activity might be a compensatory mechanism to limit excessive increases in postprandial lipemia.

Unlike some previous investigators (37, 41), we found no relation between measures of postprandial lipemia and HDL cholesterol concentration. HDL, especially HDL_2, may regulate the clearance of postprandial TG-rich particles by donating apoE and by accepting apoC peptides. Lack of this association in our study may be due to the fact that the participants were selected to have HDL cholesterol levels below 1.1 mmol/l, which corresponds to the lowest tertile of middle-aged Finnish men (42). Thus, the relative homogeneity of our patients with respect to HDL levels might have concealed the expected association.

ApoE is essential for the removal of CM remnants because it is the ligand that interacts with hepatic receptors. It is well established that apoE2 is a poor ligand compared with apoE3 or apoE4 (43). Weintraub, Eisenberg, and Breslow (44) showed that apoE3/2 subjects had higher postprandial non-CM RE levels than subjects with the 3/3 phenotype, and Boerwinkle et al. (45) confirmed this finding in a larger study. On the other hand, in agreement with our univariate analyses (Table 3), some smaller studies have not found a significant difference in the magnitude of postprandial lipemia between subjects with phenotype E2/3 and those with phenotype E3/3 (46, 47). Our multivariate analyses

TABLE 4. Multivariate linear regression analyses to predict postprandial change in trigyceride, retinyl ester, and apolipoprotein E concentrations in the chylomicron ($S_f > 400$) and in the S_f 12 to 400 fractions

	Chylomicron Triglyceride Adjusted R^2 = 0.512		Chylomicron Retinyl Ester Adjusted R^2 = 0.408		Chylomicron ApoE Adjusted R^2 = 0.434	
	SRC	*P*	SRC	*P*	SRC	*P*
Fasting TG	0.737	<0.001	0.525	<0.001	0.663	<0.001
BMI	−0.296	0.007	−0.282	0.019	−0.206	0.079
HL	0.262	0.023	0.296	0.020	0.310	0.014
ApoE2	−0.251	0.029	−0.351	0.006	−0.092	0.449
2-h glucose	0.040	0.709	0.268	0.027	−0.052	0.565
	S_f 12–400 Triglyceride Adjusted R^2 = 0.260		S_f 12–400 Retinyl Ester Adjusted R^2 = 0.194		S_f 12–400 ApoE Adjusted R^2 = 0.000	
	SRC	*P*	SRC	*P*	SRC	*P*
Fasting TG	0.519	<0.001	0.149	0.272	0.219	0.171
HL	0.165	0.215	0.364	0.011	0.101	0.538
SP24	−0.344	0.014	−0.071	0.613	−0.121	0.469
T	0.047	0.713	−0.370	0.008	0.056	0.723

Apo indicates apolipoprotein; SRC, standardized regression coefficient; TG, trigyceride; BMI, body mass index; HL, postheparin plasma hepatic lipase activity; ApoE2, presence of the ε2 allele of apoE (phenotype 3/2 or 4/2; the negative sign indicates that the presence of apoE2 is associated with smaller postprandial changes than its absence); 2-h glucose, blood glucose concentration 2 h after ingestion of 75 g of glucose; SP24, presence of the deletion allele of the apolipoprotein B signal peptide gene (the negative sign indicates that the presence of this allele is associated with smaller postprandial changes than its absence); and T, presence of the apoC-III C_{11000} to T allele.

(Table 4) did not suggest any significant role for apoE2 in predicting levels of postprandial S_f 12–400 lipoproteins. However, the statistical analyses did suggest that when fasting TG levels, BMI, and HL activity were accounted for, apoE2 was associated with low CM TG responses. This analysis is limited by the relatively small number of apoE2-positive subjects among our patients. In agreement with Boerwinkle et al. (45), we found no difference in postprandial lipemia between carriers and noncarriers of the ε4 allele. Other investigators have found a smaller (44) or larger (48) postprandial lipemic response in apoE4 subjects compared with apoE3/3 subjects. Brown and Roberts (47) reported a larger response in apoE4 subjects, but when the responses were adjusted for fasting TG levels, apoE4 predicted a lower level of postprandial lipemia. These conflicting results are probably due to differences in the sample sizes and population characteristics of the reported studies.

We studied the influence of four additional genetic polymorphisms (two of the apoB gene, LPL gene, and apoC-III gene) on postprandial lipemia. We found no associations between any of these genetic markers and postprandial lipemia in univariate analyses. In multivariate models, however, statistically significant associations were discovered between postprandial lipemia in the S_f 12–400 fraction on the one hand and apoB signal peptide length polymorphism and the apoC-III C_{1100} to T sequence change on the other.

For the apoB gene, the *XBa* I polymorphism does not alter the apoB amino acid sequence, but it is associated with differences in fasting lipid concentrations in at least some studies (reviewed in ref. 49). It has thus been hypothesized that the "X+" allele might be in linkage disequilibrium with variation in the apoB gene that affects the receptor-binding characteristics of apoB-containing lipoproteins. However, we found no difference in postprandial lipemia among individuals positive or negative for the "X+" allele (Table 2). Although CMs and their remnants contain apoB-48 that does not interact with hepatic receptors (50), apoB-100-containing particles contribute significantly to postprandial lipemia (51). Therefore, altered receptor binding of apoB-100 could potentially influence the magnitude of postprandial lipemia, and our results do not exclude the possibility that the *Xba* I polymorphism might be related to postprandial lipemia in other populations.

It was recently shown by Sturley et al. (52) in a yeast expression system that the deletion allele (SP24 allele encoding a protein containing 24 amino acids) of the apoB signal peptide is less effective in mediating the translocation of the apoB polypeptide into the endoplasmic reticulum than the wild-type 27 amino acid signal peptide (SP27). Thus, this polymorphism might influence the synthesis of intestinal and hepatic lipoproteins in the postprandial state. In support of this, in a pilot study of determinants of postprandial lipemia in young Swedish coronary patients and matched controls (3), those with the apoB signal peptide SP24 allele had

a lower degree of lipemia, and this was observed as both lower levels of remnants of intestinal (CM) and hepatic origin (53). Régis-Bailly et al. (54) found that homozygosity for the SP24 allele was related to a low postprandial response of particles containing apoB and apoC-III independently of fasting TG levels. The present study confirms this association, with those with the SP24 allele having roughly 25% lower change in S_f 12–400 lipoprotein TG levels compared to those with the SP27 allele, when the TG increments were adjusted for fasting TG levels.

Among other proteins known to be relevant to the metabolism of postprandial lipoproteins are LPL and apoC-III. We found no relation between the measures of postprandial lipemia and the *Hind* III polymorphism of the LPL gene. By contrast, Miesenböck and coworkers (55) showed that G188E, a missense mutation in the LPL gene which in vitro has no residual LPL activity, had a profound influence on postprandial lipid metabolism despite normal fasting TG levels. We also found that among our dyslipidemic patients with coronary artery disease postheparin plasma LPL activity was at best only weakly associated with measures of postprandial lipemia and was excluded from the multivariate regression models. This finding contrasts with the well-known negative relation between postprandial lipemia and LPL activity among healthy subjects (36, 37), but agrees with previous findings of the disruption of this relationship in pathological states such as non-insulin-dependent diabetes and coronary artery disease (27, 38).

We found that the C_{1100} to T sequence change in the apoC-III gene was associated with low levels of RE in the S_f 12–400 fraction. At the present time the mechanism of this association is unclear. The C–T change in the apoC-III gene occurs within the coding region of the gene, but does not alter an amino acid. In several studies, the T allele has been reported to be associated with elevated levels of plasma TG (24, 56), with higher levels of apoC-III (R. E. Peacock et al., unpublished data), and in the Swedish postprandial study (3) with a greater degree of postprandial total TG elevation (57). It has been hypothesized that the C–T change is a marker for functional change in the promotor region of the apoC-III gene that alters expression of the gene and that those with the T allele might be predisposed to have higher levels of apoC-III. This may be of biological relevance as apoC-III inhibits the removal of remnant particles (58) and results in fasting and possibly postprandial hypertriglyceridemia. The delayed clearance of intestinal lipoproteins allows for greater exchange of RE, and is seen in this study as lower RE in the S_f 12–400 fraction. However, further work in a larger sample will be needed to confirm this.

We recognize that many variables that might be relevant to postprandial lipoprotein metabolism (e.g., different sizes and compositions of the test meal, lecithin: cholesterol acyltransferase activity, apoA-IV concentration, and lipoprotein distribution of apoC-III) were not available in the present study. Nevertheless, using multivariate models based on 15 potential predictor variables describing salient demographic, metabolic, and some genetic characteristics of our study population, we were able to predict up to 50% of the variation in postprandial lipemia in the CM fraction (Table 4). Predictive power was considerably weaker for increments of TG and RE in the S_f 12–400 fraction and nonexistent for apoE. Therefore, at present, factors controlling the magnitude of postprandial lipemia in this fraction, which contains atherogenic remnants of apoB-48- and apoB-100-containing lipoproteins (59), remain elusive and require further investigation.

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