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Journal

Journal of Cardiovascular Pharmacology and Therapeutics, 6(1)

ISSN

1074-2484

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Publication Date

2001-03-01

DOI

10.1177/107424840100600106

Elevated Baseline Triglyceride Levels Modulate Effects of HMGCoA Reductase Inhibitors on Plasma Lipoproteins

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Background: The response in levels of very-low-density (VLDL) and low-density (LDL) lipoproteins varies substantially among hyperlipidemic patients during treatment with HMGCoA reductase inhibitors. Apolipoprotein E genotype and gender are known to contribute to the regulation of steady state levels of plasma lipoproteins. This study explores the effect of these and other potential determinants of the response of VLDL and LDL to treatment with reductase inhibitors.

Methods: Using mixed linear statistical models, the response of lipoprotein lipid values was studied in 142 hyperlipidemic individuals who were treated with reductase inhibitors. Patients received one or more of the following drugs individually for a total of 623 treatment observations: lovastatin, pravastatin, simvastatin, or atorvastatin. For evaluation of the effects of treatment in the aggregate, actual doses were expressed as equivalent doses of atorvastatin, using factors based on random assignment comparisons in 16 reported studies. The analysis factors considered were apolipoprotein E genotype, baseline average triglycerides >170 mg/dL (vs less), and gender.

Results: Presence of an apo ε4 allele was associated with a trend toward greater reduction of triglyceride levels and a diminished ability of the reductase inhibitors to reduce LDL cholesterol levels. Gender had only minimal effect on the response of either LDL cholesterol or triglycerides. However, the effect of elevated baseline triglycerides on the response of both triglycerides and LDL cholesterol was striking and was exerted in opposite directions. The triglyceride-lowering effect of reductase inhibitors was greater in patients with initial triglyceride levels above 170 mg/dL (P=0.0001). The effect was even greater in patients with initial triglyceride levels over 250 mg/dL (P=0.015). Conversely, for LDL cholesterol levels, elevated baseline triglycerides were associated with a significantly decreased response to the drugs (P=0.0015).

Conclusions: These findings indicate that baseline triglyceride levels are an important predictor of response of plasma lipoproteins to HMGCoA reductase inhibitors, perhaps reflecting fundamental differences in mechanism underlying the hyperlipidemic phenotype.

Key words: apo E genotype, low-density lipoproteins, very-low-density lipoproteins.

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This work was supported by an NIH grant, HL-31210; by a Grant in Aid from the American Heart Association; by a grant from Hyseq, Inc.; and was carried out in part in the General Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources, 5 MO1 RR-00079, U.S. Public Health Service.

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Polymorphisms at the apolipoprotein E (apo E) locus have been found to influence the metabolism of lipoproteins in the apo B-100 cascade. Three common alleles (ε2, ε3, and ε4), are recognized in apo E (1). The gene products, apo E-3 and E-4 but not E-2, are ligands for the low-density lipoprotein (LDL) receptor, functioning in the endocytosis of remnant lipoprotein particles formed from very-low-density lipoproteins (VLDL) and chylomicrons. Apo E-4 distributes more toward high density lipoproteins (HDL) than does apo E-3. In many, but not all studies, plasma lev-

els of LDL have been found to be higher in the presence of one or more apo E-4 alleles than in patients homozygous for ε3 alleles (2–4). Also, the risk of death from myocardial infarction is higher among individuals carrying an ε4 allele than those homozygous for ε3 alleles, even at comparable levels of LDL (5). These biological differences raise the question as to whether the apo E genotype may exert a pharmacogenomic effect on the lipoprotein response to HMGCoA reductase inhibitors. Several studies have adduced data suggesting that LDL levels among carriers of ε4 alleles are less responsive to reductase inhibitors than among individuals lacking ε4 alleles (2,6,7), whereas others have reported either no difference based on apo E genotype (5,8), or a trend that did not achieve significance (9). In this study we detected a marginally significant reduction in the response of LDL cholesterol levels to reductase inhibitors among carriers of the ε4 allele. We further found that there is a highly significant increase in the impact of reductase inhibitors on plasma triglyceride levels and VLDL cholesterol, and a reciprocal reduction in the response of LDL cholesterol levels, among individuals who have elevated baseline levels of triglycerides.

Methods

Study Design

This study was a retrospective analysis of the response of plasma lipoproteins among hyperlipidemic patients treated in the Lipid Clinic of the University of California, San Francisco. Patients were selected randomly by accession number, post hoc, for genotyping at the apo E locus. The three physicians who managed the treatment of all patients were blind to apo E genotype. Baseline lipoprotein studies were obtained when the patients had been receiving no lipid lowering medications for at least 1 month.

To detect possible drug specific effects, data on patients receiving one or more of four reductase inhibitors individually in the absence of other lipid-lowering drugs: lovastatin (263), pravastatin (82), simvastatin (177), and atorvastatin (169) were analyzed. The numbers in parentheses indicate the number of individual observations for each drug. Factors expressing equivalent dose effects in the mid-therapeutic range were calculated for the individual agents relative to atorvastatin, based on published random assignment, side by side comparisons (10–25). The factors employed in this study were: atorvastatin, 1.0; simvastatin, 0.76; lovastatin, 0.35; and pravastatin, 0.32; expressed as relative reduction in LDL cholesterol level per milligram of drug as a daily dose.

The Sample

Patients with abnormal thyroid or renal function, diabetes mellitus or any systemic disorder other than arteriosclerosis were excluded. Homozygotes for the ε2 allele were excluded because that genotype may predispose to remnant lipoprotein retention. For the study of baseline relationships of lipoprotein values to apo E status and other determinants, 288 observations on 170 individuals were analyzed.

Among these individuals, 142 (69 male and 73 female, mean ages 58.1 and 58.8 yr, respectively), had sufficient data to evaluate the effect of treatment with one or more reductase inhibitor on at least two lipoprotein measurements while taking a specific dose of reductase inhibitor. The distribution of genotypes in this subset was ε4/ε4, 7; ε4/ε3, 54; ε4/ε2, 1; ε3/ε3, 73; ε3/ε2, 7. There were 866 observations, averaging 6.1 per subject. Patients had from 1 to 4 baseline values, average 1.7. The average number of observations on each dose of an individual drug was 2.1.

Lipoprotein Studies

Lipoprotein measurements were made at every visit. Blood was drawn after a 10-hr fast for ultracentrifugal separation of the $d<1.006$ g/cm^3 and $d>1.006$ g/cm^3 fractions (26). High-density lipoprotein cholesterol was measured after precipitation of apo-B–containing lipoproteins with dextran sulfate and magnesium (27). Cholesterol and triglyceride levels were measured in plasma and in lipoprotein fractions by an automated fluorescence method (28). LDL cholesterol was calculated as the difference of the content of the LDL plus HDL fraction ($d<1.006$ g/cm^3) and the plasma HDL cholesterol, measured after precipitation of apo-B–containing lipoproteins.

Determination of Apo E Genotype

The genotype was determined using the sequencing by hybridization technique (SBH) (29,30) in the laboratories of Hyseq, Inc., Sunnyvale, CA. The accuracy of the assignment of genotype by SBH was determined by retesting a random subset of samples using an RFLP technique (31).

For each pair of primers, at least 35 polymerase chain reaction (PCR) cycles were performed using either AmpliTaq DNA polymerase at 0.025 U/μL (PE Biosystems, Foster City, CA) or cloned pfu DNA polymerase at 0.0083 U/μL in pfu PCR buffer. Specific PCR conditions were 40 to 42 cycles at 94°C

for 15 seconds, 55°C for 15 seconds, and 72°C for 90 seconds using 3 to 10 ng/μL of primer.

Sequencing by hybridization is a sequencing process in which a set of overlapping probes is scored for hybridization to a target DNA on a solid support (29). Polymerase chain reaction samples were dispensed from microtiter plates to 22.5 × 15.3-cm Genescreen nylon membranes (New England Nuclear, Boston, MA) using a gridding robot with 3-axis gantry equipped with a pin tool which transfers 20 nL to each spot, achieving a total DNA mass of 0.5 ng per spot. Membranes were washed for at least 10 seconds in 0.5 M NaOH solution to denature DNA samples, dried for 3 hours at room temperature, and irradiated at 1200 J in a UV-crosslinker (Stratagene, La Jolla, CA) to fix the DNA. In this experiment, 42 to 46 sample clones were arrayed in 64 spot subarrays, with a total of 384 identical subarrays; on each membrane. The positive and negative controls and markers are included (30).

Unlike SBH, some hybridization procedures (for instance ASO, allele-specific oligonucleotide assay) rely on only a single probe to identify each allele. In SBH, several overlapping probes are used to determine the identity of each base. With 7-mers, up to 14 different probes (seven per strand) are used to identify each base pair, minimizing the impact of false-positive or false-negative hybridization results. Probes (Biosource [Palo Alto, CA] or GenSet [San Diego, CA] used in SBH experiments were individual oligomers or pools consisting of specific oligomer cores surrounded by two to four variable bases, for example, N-B7-NN. (N represents any of the four DNA bases.) Hence a 7-mer probe with three variable bases consists of a pool of 64 possible 10-mers, each with an identical 7-mer core. The variable bases elongate the probe, increasing the thermodynamic stability of the DNA-probe hybrid and improving discrimination. Ten nanograms of each probe were labeled with 0.125 μL γ^{33}P-ATP (10 μCi/μL) (Amersham, Piscataway, NJ) in 384-well plates (NUNC or CoStar) using 0.05 μL T4 polynucleotide kinase (30 units/μL) (Amersham, Piscataway, NJ) and 2.0 μL 10X kinase buffer.

Hybridization buffer and aliquots of probe (0.8 pmol/mL) were added to each chamber using a Biomek Automated Laboratory Workstation (Beckman Instruments, Fullerton, CA) equipped with a pipetting tool. Hybridization buffer was 0.2 M sodium phosphate and 3% lauryl sarcosine. After hybridization, membranes were washed in cold 4× SSC (0 to 5°C for 30 min.

Membranes were blotted on filter paper and placed in cassettes containing phosphor screens (Molecular Dynamics, Sunnyvale, CA) for 1 hr at 4°C before reading by PhosphorImager® scanner (Molecular Dynamics). The following format 1-SBH image analysis programs were used in data analysis:

- Image analysis (flanew): locates the physical position of probes in the array and assigns a signal intensity (raw score) to each probe.
- Mass normalization (MassNorm): compensates for mass differences between different sample spots in an array.
- Sequence analysis (ida): deselects probes and/or samples based on statistical analysis of normalized scores; calculates positional scores (for each sample and position in the reference sequence calculates a score indicating whether the base at that position is wild-type, heterozygote, or homozygote); determines the most probable base for each sample and position.

Statistical Methods

The data analysis employed SAS version 6.12 (32,33). The REG procedure was used to fit models on data for individual subjects, to obtain diagnostic measures on each observation (deleted residuals, Cook's distance, DFFITS) for data-verification purposes. Procedure GLM was used for preliminary modeling to obtain adjusted (least squares) means. Random-coefficient mixed linear models were fitted using the MIXED procedure. Among candidate models for a given response, the selected one maximized the bias-corrected Akaike information criterion (CAIC) based on the total number of model parameters (variance components plus fixed effects) (34). The CAIC was used in the form without the multiplier –2, so that larger values indicated better model adequacy.

Results

Genotyping at the Apo E Locus

The genotype assignment at the apo E locus was made using SBH (29). To test the accuracy of this technique, a sample of 465 randomly selected genomic DNA samples were genotyped by SBH and by an established RFLP technique (30). The assignment of genotype was congruent in 464 samples. The remaining sample, genotyped as ε2/ε2 by RFLP was assigned an ε2/ε3 genotype by SBH, yielding an error rate of 0.22%.

Baseline Characteristics of the Patient Cohort

Subject means of baseline lipoprotein values for the patient cohort are shown in Table 1. Baseline values

Table 1. Subject Means of Baseline Average Lipoprotein Values

Variable	Mean (mg/dL)	SD
Total triglycerides	218.2	149.0
Total cholesterol	299.7	72.4
LDL cholesterol	209.4	69.5
HDL cholesterol	48.6	15.9

were analyzed with respect to three factors: Apo E4 status (present; absent), baseline average triglycerides (TG) status (>170 mg/dL; ≤ 170 mg/dL), and gender. In the analysis models, corresponding factor variables APOE4 (presence of one or more ε4 alleles), high baseline triglycerides (HIGHBSTG), and FEMALE were utilized, each having the value 1 when the named condition was present and 0 when it was absent; e.g. HIGHBSTG=1 if baseline average TG exceeded 170 mg/dL. The value of 170 mg/dL for triglycerides was chosen a priori on clinical grounds as a discriminant. Mixed linear models were used. Nesting of observations within subjects was specified, and the subject-specific intercept was the random effect used. For each of the variables: TG, log base 2 of triglycerides (LG TG), LDL cholesterol (LDL-C), and log base 2 of LDL cholesterol (LG LDL-C, a model with all interaction effects of the factors and a model with only additive effects were compared. For TG and LDL-C, the all-interaction-effects models were better according to the model adequacy criterion, whereas the additive models were appropriate for LG TG and LG LDL-C. For brevity and clarity, we summarize only the results for the additive models (see Discussion).

For baseline LG TG, there were no significant effects except, predictably, for baseline average TG status. For baseline LG LDL-C, subjects with ε4 alleles had higher adjusted mean levels (7.746 for ε4 vs 7.556 for non ε4, P=0.012). Those with baseline average TG levels over 170 mg/dL had lower adjusted mean levels of LG LDL-C (7.563 vs 7.750, P=0.008), and females had higher adjusted mean levels of LG LDL-C (7.7774 vs 7.539, P=0.001).

Preliminary Analyses of Response of LDL-C and TG to Individual Reductase Inhibitors

In some preliminary analyses, we examined absolute and relative (percentage) changes in LDL-C and TG from their baseline averages, in response to individual drugs on doses of a given reductase inhibitor that were taken by at least 10 subjects. In an attempt to standardize the change scores over different doses, subject mean change scores at each dose were further divided by the dose to obtain change per milligram of reductase inhibitor.

In examination of these change scores, each dose of each reductase inhibitor was analyzed separately with respect to first-order models in APOE4, HIGHBSTG, and FEMALE, for a total of 12 reductase inhibitor-dose combinations. Adjusted means for the change scores were obtained for each of the factors, and compared for statistical significance between factor levels. Table 2 summarizes the results for comparisons that were significant at P<0.1. N denotes the number of subjects at the given drug and dose level in this analysis. Absolute change scores are labeled ABSCHG and relative change scores, RELCHG. For each factor, MEAN0 denotes the adjusted mean at level 0 of the factor (indicating absence of the effect), while MEAN1 denotes the adjusted mean when the effect was present. LOW indicates which factor level had the lower adjusted mean and hence the greater magnitude of average decrease from baseline. Three drug-dose combinations in this analysis did not produce any comparisons significant at the 0.1 level: atorvastatin at 10 mg (N=18), pravastatin at 20 mg (N=12), and simvastatin at 20 mg (N=16).

On the 12 tests for each factor, response variable and type of change score (absolute or relative), the number of tests expected to be significant at 0.1 by chance alone is 1.2. For the effect of APOE4 on LDL-C, there was just one significant difference for each type of change score (for pravastatin at 40 mg), which is at the chance level of frequency. For the effect of APOE4 on TG, the levels with lower means were mixed for lovastatin, but consistent for atorvastatin, with Apo E4 being associated with significantly lower means at two of the three dose levels studied. This suggests that for subjects receiving atorvastatin, those with the Apo E4 allele tended to experience a greater decrease in TG per milligram of reductase inhibitor.

With respect to HIGHBSTG, subjects with lower baseline average TG (<=170 mg/dL) tended to experience a greater absolute decrease in LDL-C levels per milligram of reductase inhibitor, as compared to subjects with high baseline average TG. Primarily, this was evident for subjects on lovastatin. Further, there was a strong suggestion that subjects with high baseline average TG tended to experience greater decreases in TG per unit dose. This effect was evident for all of the reductase inhibitors studied, except for pravastatin. Further, in a supplemental analysis, the greatest absolute changes in TG tended to occur in patients with baseline TG ≥ 250 mg/dL, again except for those receiving pravastatin.

Table 2. Adjusted Means of Lipid Change Scores for APOE4, HIGHBSTG and FEMALE; $P<0.1$ for Difference Between Levels

Statin	STDose	N	Score	Mean0	Mean1	Low	P
Factor=APOE4 RESPONSE=LDL-C							
PRAVA	40	14	ABSCHG	-2.41	-0.98	0	0.0491
PRAVA	40	14	RELCHG	-0.97	-0.47	0	0.0907
Factor=APOE4 RESPONSE=TG							
ATOR	20	21	ABSCHG	-1.47	-5.70	1	0.0843
ATOR	40	24	ABSCHG	-0.77	-2.85	1	0.0720
ATOR	20	21	RELCHG	-0.47	-1.63	1	0.0688
ATOR	40	24	RELCHG	-0.22	-0.96	1	0.0192
LOVA	20	20	RELCHG	-0.72	-1.64	1	0.0544
LOVA	40	48	RELCHG	-0.40	-0.00	0	0.0562
Factor=HIGHBSTG RESPONSE=LDL-C							
ATOR	40	24	ABSCHG	-3.21	-1.97	0	0.0716
LOVA	20	20	ABSCHG	-2.98	-1.84	0	0.0839
LOVA	40	48	ABSCHG	-2.29	-1.27	0	0.0011
LOVA	60	22	ABSCHG	-2.35	-1.41	0	0.0453
LOVA	40	48	RELCHG	-0.89	-0.63	0	0.0109
Factor=HIGHBSTG RESPONSE=TG							
ATOR	20	21	ABSCHG	0.41	-7.59	1	0.0071
ATOR	40	24	ABSCHG	-0.71	-2.90	1	0.0666
LOVA	20	20	ABSCHG	-0.74	-4.06	1	0.0006
LOVA	40	48	ABSCHG	0.10	-1.55	1	0.0207
SIMVA	10	11	ABSCHG	0.40	-14.01	1	0.0909
SIMVA	40	31	ABSCHG	-0.46	-2.99	1	0.0198
ATOR	20	21	RELCHG	-0.17	-1.93	1	0.0192
LOVA	20	20	RELCHG	-0.57	-1.79	1	0.0104
LOVA	40	48	RELCHG	0.06	-0.47	1	0.0095
LOVA	80	13	RELCHG	-0.42	-0.08	0	0.0817
SIMVA	10	11	RELCHG	-0.95	-4.36	1	0.0278
Factor=FEMALE RESPONSE=LDL-C							
LOVA	20	20	ABSCHG	-1.85	-2.97	1	0.0725

For females vs males, there was only one significant test among 48, which is below the frequency expected by chance. Hence, gender may not be a significant determinant of response.

Modeling the Response of LDL-C and TG to Four Reductase Inhibitors, in the Aggregate, Adjusted to Equivalent Doses

For combined analyses, doses of the reductase inhibitors were converted to equivalent doses of atorvastatin (EQDOSE), as indicated in Methods. Values of EQDOSE were rounded to one decimal place. In the 142 subjects, the 623 non-baseline values of EQDOSE averaged 21.43 and ranged from 3.2 to 80. A total of 866 observations were analyzed, 243 of which were baseline.

Mixed linear models, having both fixed and random effects, were fitted to the response variables. In addition to LDL-C and TG, the logarithms (base 2) of these variables, LG LDL-C and LG TG, were analyzed so that both additive and multiplicative effects could be assessed. Fixed effects included an intercept, APOE4, HIGHBSTG and FEMALE, plus EQDOSE or some transform of this variable. The random effects were INTERCEPT and identically-transformed EQDOSE, which modeled subject-specific deviations from the corresponding fixed effects.

Transforms considered for EQDOSE were EQDOSE itself, RTEQDOSE=square-root (EQDOSE) and LGEQDOS1=log base 2 (EQDOSE + 1), which all equal 0 when EQDOSE=0. The minimal fixed-effects models included the factors APOE4, HIGHBSTG and FEMALE, plus transformed EQDOSE, plus the product of transformed EQDOSE with each of the preceding factors. Relative to these minimal models, linearity of response with respect to the various transforms of EQDOSE was checked by adding a quadratic term in the same transform (eg, for the model using EQDOSE, EQDOSE*EQDOSE was added). Adequacy of the minimal model, as measured by the CAIC criterion, was most degraded by addition of the quadratic term when LGEQDOS1 was used in models for TG, LG TG, and LDL-C, and when RTEQDOSE was used in the model for LG LDL-C. The *P*

value for the quadratic term in the selected transform was 0.17 for LG LDL-C, and greater than 0.4 for the other responses. In all instances, minimal models utilizing the selected transform had better model adequacy values (and greater values of the likelihood) than corresponding models using EQDOSE.

To select a final analysis model for each response, two other models were considered: a model on APOE4, HIGHBSTG, FEMALE and the selected transform of EQDOSE that included all interaction effects through second order (three-term products); and a similar model that included interaction effects through first order only. Among the three choices, the most adequate model for TG included all first-order interactions, while the minimal models were most adequate for LG TG, LDL-C, and LG LDL-C. For brevity and clarity, only results for response to reductase inhibitors for the latter three models will be presented.

Results From the Models

Tables 3 to 5 summarize the estimated regression coefficients for the fixed effects in these models. In addition, the fitted linear models for each combination of factor levels are presented, as well as the predicted means when EQDOSE=80, the maximum value.

For LG TG, the effect of HIGHBSTG*LGEQDOS1 was negative (effect=–0.088, P=0.0001), indicating that subjects with high baseline average TG tended to have a steeper decline in LG TG with increasing dose. An analogous result was obtained for untransformed TG. In a supplemental analysis in which an additional category of average baseline TG was represented (XHIBSTG=1 if average baseline TG ≥ 250 mg/dL; = 0 otherwise), the effect of XHIBSTG* LGEQDOS1 was negative (effect=–0.062, P=0.015) as was the effect of HIGABSTG*LGEQDOS1 (effect=–0.049, P=0.036). This indicated that among the 39 subjects with very high baseline TG, on average an even steeper decline in LG TG occurred with increasing doses.

In the model for LDL-C, the effect of LGEQDOS1 was negative (effect=–21.5, P=0.0001) and indicated a strong dose response for males without Apo E4 and with lower baseline average TG. However, HIGHBSTG*LGEQDOS1 had a positive effect (effect=5.1, P=0.0015), indicating that subjects with high baseline average TG tended to have a less steep decline in LDL-C with increasing dose.

For LG LDL-C, the effect of RTEQDOSE was negative (effect=–0.185, P=0.0001), indicating a strong decline in LG LDL-C levels with increasing dose for males without Apo E4 and with lower baseline average TG. Here, the effect of APOE4*RTEQDOSE was positive (effect=0.023, P=0.059), suggesting that subjects with Apo E4 had a less steep decline with increasing dose.

Finally, to more closely parallel the results of Table 2, we carried out mixed model analyses separately for each of the four reductase inhibitors. In the analysis for each drug, for each subject who had observations

Table 3. Fixed-Effects Model for LG TG

Effect	Estimate	Standard Error	DF	*P*
INTERCEPT	6.949	0.081	138	0.0001
APOE4	–0.065	0.086	582	0.4521
HIGHBSTG	1.299	0.085	582	0.0001
FEMALE	–0.160	0.085	582	0.0606
LGEQDOS1	–0.044	0.017	138	0.0116
APOE4*LGEQDOS1	0.006	0.018	582	0.7605
HIGHBSTG*LGEQDOS1	–0.088	0.018	582	0.0001
FEMALE*LGEQDOS1	–0.004	0.018	582	0.8452

Fitted Model for Each Combination of Factor Levels

APOE4	HIGHBSTG	FEMALE	Intercept	Slope on LGEQDOS1	Predicted at EQDOSE=80
0	0	0	6.949	–0.044	6.668
0	0	1	6.789	–0.048	6.486
0	1	0	8.248	–0.133	7.407
0	1	1	8.088	–0.136	7.224
1	0	0	6.885	–0.039	6.639
1	0	1	6.724	–0.042	6.456
1	1	0	8.183	–0.127	7.377
1	1	1	8.023	–0.131	7.194

Table 4. Fixed-Effects Model for LDL-C

Effect	Estimate	Standard Error	DF	*P*
INTERCEPT	211.5	10.6	138	0.0001
APOE4	21.8	11.4	582	0.0560
HIGHBSTG	–35.1	11.3	582	0.0019
FEMALE	23.8	11.3	582	0.0349
LGEQDOS1	–21.5	1.5	138	0.0001
APOE4*LGEQDOS1	–0.0	1.6	582	0.9961
HIGHBSTG*LGEQDOS1	5.1	1.6	582	0.0015
FEMALE*LGEQDOS1	–0.4	1.6	582	0.7949

Fitted Model for Each Combination of Factor Levels

APOE4	HIGHBSTG	FEMALE	Intercept	Slope on LGEQDOS1	Predicted at EQDOSE=80
0	0	0	211.5	–21.5	75.5
0	0	1	235.3	–21.9	96.7
0	1	0	176.4	–16.3	72.9
0	1	1	200.2	–16.7	94.0
1	0	0	233.3	–21.5	97.3
1	0	1	257.1	–21.9	118.4
1	1	0	198.2	–16.3	94.7
1	1	1	222.0	–16.7	115.8

Table 5. Fixed-Effects Model for LG LDL-C

Effect	Estimate	Standard Error	DF	*P*
INTERCEPT	7.621	0.068	138	0.0001
APOE4	0.162	0.072	582	0.0253
HIGHBSTG	–0.210	0.071	582	0.0034
FEMALE	0.154	0.071	582	0.0311
RTEQDOSE	–0.185	0.011	138	0.0001
APOE4*RTEQDOSE	0.023	0.012	582	0.0593
HIGHBSTG*RTEQDOSE	0.009	0.012	582	0.4332
FEMALE*RTEQDOSE	0.018	0.012	582	0.1334

Fitted Model for Each Combination of Factor Levels

APOE4	HIGHBSTG	FEMALE	Intercept	Slope on RTEQDOSE	Predicted at EQDOSE=80
0	0	0	7.621	–0.185	5.968
0	0	1	7.775	–0.167	6.283
0	1	0	7.412	–0.175	5.842
0	1	1	7.566	–0.157	6.157
1	0	0	7.783	–0.162	6.334
1	0	1	7.937	–0.144	6.648
1	1	0	7.573	–0.153	6.207
1	1	1	7.727	–0.135	6.522

on that drug, all baseline observations were included. The model used for each response was as above. We report the significant ($P<0.1$) interaction effects of the selected EQDOSE transform with APOE4, HIGHBSTG and FEMALE, in the same order as for Table 2.

The effects of APOE4 on the response of LDL-C or LG LDL-C to reductase inhibitors were as follows: with pravastatin, both the effects of APOE4*LGEQDOS1 (effect=8.2, $P=0.029$) on LDL-C and APOE4*RTEQDOSE (effect=0.101, $P=0.012$) on LG LDL-C were positive. This indicates a less steep decline in LDL-C for subjects with Apo E4 who received pravastatin, paralleling the Table 2 result.

Effects of APOE4 on the response of TG or LG TG to reductase inhibitors were: with atorvastatin, the effect of APOE4*LGEQDOS1 was negative for both

responses but marginally significant for TG (effect=7.7, P=0.098) and not for LG TG (effect=–0.029, P=0.28). This indication of a more steep decline in TG for subjects with Apo E4 who received atorvastatin partially parallels the Table 2 result. With lovastatin, the effect for LG TG was positive but nonsignificant (P=0.32), which reasonably resolves the inconsistency of the Table 2 result for lovastatin.

Effects of HIGHBSTG on the response of LDL-C or LG LDL-C to reductase inhibitors: The effect of HIGHBSTG*LGEQDOS1 was positive for LDL-C with atorvastatin (effect=4.7, P=0.074) and lovastatin (effect=8.9, P=0.0008), while the effect of HIGHBSTG-RTEQDOSE was positive for LG LDL-C with lovastatin (effect=0.042, P=0.017). These indications of a less steep decline in LDL-C for subjects with high baseline average TG are consonant with Table 2 results.

Effects of HIGHBSTG on the response of TG or LG TG to reductase inhibitors: the effect of HIGHBSTG*LGEQDOS1 was negative for TG with atorvastatin (effect=23.5, P=0.0001), lovastatin (effect=–14.0, P=0.005), and simvastatin (effect=–23.7, P=0.0003). Also, the effect was negative for LG TG with atorvastatin (effect=–0.093, P=0.0008), lovastatin (effect=–0.062, P=0.028), and simvastatin (effect=–0.099, P=0.0012). These indications of a steeper decline in TG for subjects with high baseline average TG again parallel those of Table 2.

Effects of female gender on the response of LDL-C or LG LDL-C to reductase inhibitors: with atorvastatin, the effect of FEMALE*RTEQDOSE on LG LDL-C was positive (effect=0.037, P=0.083), suggesting a less steep decline in LDL-C for females with atorvastatin. This does not correspond to the Table 2 result. There were no significant effects of FEMALE on the response of TG or LG TG to reductase inhibitors.

In total, among tests on interaction effects with three different factors on four response variables for each of four reductase inhibitors (48 tests), 13 tests were significant at the 0.1 level, well above the 4.8 expected by chance.

Response of VLDL Cholesterol and VLDL Triglyceride to HMGCoA Reductase Inhibitors

Following the approach above, we fitted mixed linear models for the response of LG VLDL-C=Lg2 (VLDL-C) and LG VLDL-TG=Lg2 (VLDL-TG) to reductase inhibitors, where Lg2 denotes log base 2. As subject-specific slopes of the lipid measures vs EQDOSE or a transform were required, subjects were excluded from the analysis of a given measure unless there were observations on at least two different values of EQDOSE (possibly including 0). The minimal fixed-effects model above was adopted, including the factors APOE4, HIGHBSTG and FEMALE, transformed EQDOSE, and the product of that transform with each of the factors. Transforms of EQDOSE were examined as before, and LGEQDOS1=Lg2 (EQDOSE + 1) provided the best fit for both models.

For VLDL-C, 73 subjects passed the exclusion condition, with a total of 321 observations. In this subset of subjects, 42% were carriers of the Apo E4 allele, 47% had high baseline average TG, and 52% were female. VLDL-C ranged in value from 1 to 213 with a mean of 30, while LG VLDL-C ranged from 0 to 7.73 with a mean of 4.56. Subjects with high baseline average TG had a higher estimated mean value of LG VLDL-C at zero dose (effect=1.88, P=0.0001), and a steeper decline relative to LGEQDOS1 (effect=–0.18, P=0.0008). Also, females had a lower estimated mean value of LG VLDL-C at zero dose (effect=–0.48, P=0.024), and showed a nonsignificant tendency toward a less steep decline with respect to LGEQDOS1 (effect=0.08, P=0.13).

However, for VLDL-TG, only 31 subjects passed the exclusion condition, with a total of 92 observations. In this subset of subjects, 45% were carriers of the Apo E4 allele, 39% had high baseline average TG, and 52% were female. VLDL-TG ranged in value from 7 to 790 with a mean of 104, while LG VLDL-TG ranged from 2.81 to 9.63 with a mean of 6.36. Subjects with high baseline average TG had a higher estimated mean value of LG VLDL-TG at zero dose (effect=1.37, P=0.0001). All other effects had P>0.5, to which the small sample size undoubtedly contributed.

Discussion

The need to include interaction effects in the models probably reflects the highly skewed distributions of values for these variables, particularly TG, in which a relatively few extreme values were likely to be very influential. After log transformation, better fitting models with additive factor effects were obtained, simplifying interpretation. This obviated the need for models utilizing interaction effects for TG and LDL-C in the baseline analyses and for triglyceride in the analyses of response to drugs. The retrospective design of the study could potentially introduce biases with respect to patient selection or compliance.

However, the random selection of patients from a large lipid clinic population would be expected to yield a patient cohort representative of patients with moderately severe primary hyperlipidemia. The striking significance of the effect of high baseline triglycerides on both VLDL and LDL is consistent with a real biological mechanism.

Studies to date designed to detect an effect of the apo ε4 allele on the response of LDL cholesterol levels to HMGCoA reductase inhibitors have yielded equivocal results. A study of 189 French Canadian patients with heterozygous familial hypercholesterolemia showed a highly significant reduction in the response of LDL-C to lovastatin in carriers of an ε4 allele (35), whereas other studies of similar size in the U.S. and Holland failed to detect any effect attributable to ε4 alleles, (8) or a marginal effect (9). However, a meta-analysis combining data from four studies detected a significantly diminished response of LDL-C (6).

We found a significant association of apo E4 status with LDL cholesterol response with pravastatin as a single agent. When data on all four reductase inhibitors were analyzed in the aggregate, a relationship was not evident with LDL-C per se, though the relationship with LG LDL-C closely approached significance (P=0.059). This is concordant with the results of the study of a subset of 478 patients from the Scandinavian Simvastatin Survival Study (5). The ethnic admixture phenomenon could be the basis for the lack of congruity of results from the different studies, given the diverse populations involved.

A far more striking finding in this study is the highly significant effect of elevated baseline triglyceride levels on the response of triglyceride-rich lipoproteins to reductase inhibitors. Clearly, individuals with elevated baseline triglycerides experienced a markedly greater reduction in triglyceride levels per milligram of drug, an effect that was also seen in the LG transformation of triglyceride levels. This is consistent with a meta-analysis of several trials of reductase inhibitors in which subjects with a high baseline triglyceride level had a greater fractional reduction in plasma triglyceride levels than did normotriglyceridemic subjects (23).

A further finding in the present study is a highly significant opposite or positive effect of high baseline triglyceride levels on the response of LDL cholesterol levels. That is, whereas reductase inhibitors have a greater effect in reducing triglyceride levels in individuals with high baseline triglycerides, they are unable to effect reduction of LDL-C levels as well as they do in individuals with lower plasma triglycerides. Examination of the tabular data of Stein and associates (23), in a study directed at other questions, indeed reveals a diminished relative effect of reductase inhibitors on LDL cholesterol among hypertriglyceridemic patients in concordance with the observation in this study. The mechanisms underlying these observations are not readily apparent. A direct effect of reductase inhibitors on VLDL secretion (36,37) could account for the increased impact of the drugs on triglyceride levels, but not the diminished effect on LDL cholesterol levels, among hypertriglyceridemic individuals.

Because the reductase inhibitors increase expression of the hepatic LDL receptors, which endocytose remnant lipoproteins formed from VLDL, the most likely mechanism for the reduction in plasma triglyceride levels should be increased uptake of those particles by the liver. However, this should deplete the pool of intermediate density lipoproteins that are the immediate precursors of LDL, reducing the production rate of LDL and, consequently the steady state levels of LDL in blood. The diminished impact of reductase inhibitors on LDL in individuals with high baseline triglycerides thus requires another explanation. Perhaps rapid intake of cholesterol-rich VLDL remnants, reflecting the large pool available for endocytosis, loads hepatocytes with cholesterol, partially overcoming the effect of the reductase inhibitors on the transcriptional upregulation of receptors. An interesting possibility is that dichotomization of the patient cohort on the basis of baseline triglyceride levels partially separates two groups with biologically different mechanisms underlying their hyperlipidemia: primary hypercholesterolemias, and a group perhaps largely comprised of the phenotypic disorder termed familial combined hyperlipidemia. Thus, the differential response of both triglyceride-rich lipoproteins and of LDL may be a reflection of the fundamental metabolic differences in these phenotypes.

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