Effect of Statin Therapy on Remnant Lipoprotein Cholesterol Levels in Patients With Combined Hyperlipidemia

Daniel T. Stein, Sridevi Devaraj, David Balis, Beverley Adams-Huet, Ishwarlal Jialal

Abstract—Clinical trials with statins have demonstrated significant reductions in cardiovascular events. Remnant lipoproteins are independent predictors of cardiovascular events. Because of the paucity of data on the effect of statins on remnant lipoproteins, we tested the effect of pravastatin, simvastatin, and atorvastatin on remnant lipoprotein cholesterol (RLP-C) levels in a randomized crossover study in patients with combined hyperlipidemia. After a 6-week diet phase, patients (n=22) were randomized to pravastatin (40 mg/d), simvastatin (20 mg/d), or atorvastatin (10 mg/d) for 6 weeks, with a 3-week washout between each drug. All 3 drugs significantly decreased total and low density lipoprotein (LDL) cholesterol (P<0.001). Mean reduction in LDL cholesterol with pravastatin, simvastatin, and atorvastatin was 21%, 29%, and 32%, respectively. None of the drugs affected high density lipoprotein cholesterol levels. Median levels of triglycerides were significantly reduced with simvastatin (26%, P=0.001) and atorvastatin (24%, P=0.0001) but not with pravastatin (9%, P=0.18). Non–high density lipoprotein cholesterol decreased significantly with all 3 statins (20%, 29%, and 32% with pravastatin, simvastatin, and atorvastatin, respectively; P<0.001). Median RLP-C levels were significantly reduced with simvastatin (6%, P<0.05) and atorvastatin (25.9%, P<0.001) but not with pravastatin (2.9%, P=0.58). Thus, atorvastatin and simvastatin, in addition to reducing LDL cholesterol and triglyceride levels, significantly reduced RLP-C levels. This could be another potential mechanism to explain their cardiovascular benefits. (Arterioscler Thromb Vasc Biol. 2001;21:2026-2031.)

Key Words: statins ■ non-HDL cholesterol ■ remnants

Classic concepts of the pathogenesis of coronary artery disease (CAD) have implicated elevated cholesterol, particularly LDL cholesterol (LDL-C), as the central atherogenic lipoprotein. Recently reported large cholesterol-lowering trials using the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have unequivocally established their benefit in reducing cardiovascular events and coronary and total mortality in certain studies.1–6 Although the benefit of statin therapy has generally been ascribed to reduction in LDL-C, other atherogenic classes of lipoproteins may be beneficially affected by statin therapy. This is particularly pertinent because the majority of patients with CAD have only mildly elevated cholesterol levels and frequently have other lipoprotein abnormalities, including hypertriglyceridemia and decreased levels of HDL cholesterol (HDL-C).

Accumulating data suggest that apoB-containing lipoproteins other than LDL, particularly in the setting of mild to moderate hypertriglyceridemia, confer additional atherogenic risk beyond that due to LDL-C levels alone.7 Triglyceride-rich remnant lipoproteins (RLPs) are formed in the circulation when apoB-48, containing chylomicrons of intestinal origin, or apoB-100, containing VLDL of hepatic origin, are converted by lipoprotein lipase (and to a lesser extent by hepatic lipase) into smaller and more dense particles. Compared with their nascent precursors, the remnants are depleted of triglycerides, phospholipids, and apoCs and are enriched in cholesteryl esters and apoA and are believed to be more atherogenic than the larger triglyceride-rich lipoproteins (TRLs).8–12

Several lines of evidence have implicated RLPs as playing an etiologic role in atherosclerosis.13 Increased IDL levels have been associated with an increased incidence or recurrence of CAD.14 Increased IDL levels are also found in diseases associated with premature or accelerated atherosclerosis, such as type III hyperlipidemia, type 2 diabetes mellitus, chronic renal failure, and familial combined hyperlipidemia.15 In fact, the recent ATP III panel identifies non–HDL-C as a secondary target of therapy in persons with high triglycerides (>200 mg/dL), because this is a readily available measure of atherogenic RLPs.16 Accurate quantification of plasma remnants is difficult because (1) they are difficult to differentiate from their triglyceride-rich precursors, (2) as a result of rapid catabolism, they are present in plasma at low
concentrations, and (3) being at various stages of catabolism, they are heterogeneous in size, density, and composition.\textsuperscript{8} Measurement of remnant particles, particularly IDL, is not routinely performed because of the necessity for specialized testing, such as ultracentrifugation or capillary isotachopheresis. Recently, an immunoaffinity chromatography method was introduced for assaying levels of RLPs according to their apolipoprotein content and immunospecificity.\textsuperscript{8,17} In this assay, RLPs are separated from plasma by immunoaffinity chromatography with a gel containing an anti–apoA-1 and a specific apoB-100 monoclonal antibody (JI-H). The former antibody recognizes all HDL and any newly synthesized chylomicrons containing apoA-1, whereas the latter antibody recognizes all apoB-100–containing lipoproteins, except for certain particles enriched in apoE. HDLs, LDLs, large chylomicrons, and most VLDLs are thus retained by the gel. The unbound RLPs are made up of remnant-like VLDLs containing apoB-100 and TRLs containing apoB-48. By use of this assay, plasma concentrations of RLP cholesterol (RLP-C) have been shown to be higher in patients with CAD, in diabetic patients, in fed patients versus fasted patients, in hemodialysis patients, in patients with coronary artery restenosis after angioplasty, and in patients experiencing sudden cardiac death. Increased RLP-Cs are a significant predictor of myocardial infarction in patients with vasospastic angina and have recently been shown to be strongly associated with angiographically verified progression of focal coronary atherosclerosis.\textsuperscript{18–25} The atherogenicity of RLP is supported by the observations that RLP can promote lipid accumulation by mouse peritoneal macrophages, stimulate whole-blood platelet aggregation, and impair endothelium-dependent vasorelaxation.\textsuperscript{8} Thus, this assay is a valid measure of RLPs that are proatherogenic.

Current concepts of lipoprotein metabolism suggest that remnant particles are taken up by a receptor-mediated process in the liver.\textsuperscript{26} Furthermore, the LDL receptor is upregulated by inhibition of HMG-CoA reductase and leads to the potent LDL-lowering effect associated with statins. However, there is a paucity of data comparing the effect of statins on RLPs. Thus, we tested the effect of 3 widely used statins (pravastatin, simvastatin, and atorvastatin) on RLP-C levels at doses resulting in similar reductions in LDL-C.\textsuperscript{27}

Methods

Patients

Patients were recruited from the Lipid Clinics at the University of Texas Southwestern Medical Center, Dallas, without restriction to sex or socioeconomic status. The protocol was approved by the Institutional Review Board, and all patients gave informed consent. Inclusion criteria included the following: age 18 to 70 years, plasma triglyceride level between 160 and 600 mg/dL, and LDL-C level \( \geq 130 \) mg/dL. Exclusion criteria were as follows: use of lipiddowering drugs or drugs known to affect lipid metabolism within 6 weeks of the study start, antioxidant supplements, warfarin/heparin for the past 4 weeks, liver or renal dysfunction, diabetes, hypothyroidism, infection, cancer, and recent major surgery or illness.

Study Design

This was a randomized double-blind triple-crossover study. A total of 22 patients were enrolled. There was a 6-week lead-in dietary phase when the patients were instructed by the dietitian to follow an American Heart Association step-1 diet for the study duration, followed by a 6-week drug-therapy phase with a 3-week washout period between drugs. The statins used included pravastatin (40 mg/d), simvastatin (20 mg/d), and atorvastatin (10 mg/d).\textsuperscript{26}

Laboratory Methods

Three fasting blood samples were obtained at baseline 5 days apart, and 2 fasting blood samples were obtained during each drug phase (weeks 5.5 and 6) and at the end of each washout phase (weeks 8.5 and 9). Levels of total cholesterol, total triglycerides, LDL-C, and HDL-C were assayed by routine laboratory techniques with the use of methodology of the Lipid Research Clinics, as reported previously.\textsuperscript{23} If plasma triglycerides were \( \geq 400 \) mg/dL, LDL-C was assessed by a direct method.\textsuperscript{29} Only 3 of the 22 patients studied had baseline triglyceride levels \( \geq 400 \) mg/dL. ApoR was quantified by using immunonephelometry. The assay is standardized to the World Health Organization reference material.

RLP-C Assay

In this assay, RLPs are separated from plasma by immunoaffinity chromatography with a gel containing an anti–apoA-1 and a specific apoB-100 monoclonal antibody (JI-H). The former antibody recognizes all HDLs and any newly synthesized chylomicrons containing apoA-1, whereas the latter antibody recognizes all apoB-100–containing lipoproteins, except for certain particles enriched in apoE. HDLs, LDLs, large chylomicrons, and most VLDLs are thus retained by the gel. The unbound RLPs are made up of remnant-like VLDLs containing apoB-100 and TRLs containing apoB-48. These RLPs are quantified by measuring cholesterol enzymatically in the unbound fraction.\textsuperscript{8,17}

For the RLP-C assay, 300 \( \mu L \) of the immunoaffinity gel containing antibodies to apoA-1 and apoB-100 was pipetted into separation cups containing steel balls and placed in a magnetic mixer manufactured by Otsuka Electronics. After the gel was allowed to settle for 5 minutes, 5 \( \mu L \) of blank (buffer), control, or plasma was pipetted onto the surface of the gel and incubated with continuous mixing for 2 hours at room temperature. After incubation, the gel was allowed to settle for 15 minutes, and 200 \( \mu L \) of the supernatant was placed into sample cups. Cholesterol levels were then measured by using a peroxidase-based assay on the Cobas Mira S autoanalyzer (Roche Diagnostic Systems). The intra-assay and interassay coefficients of variation (<5%) have been previously reported.\textsuperscript{29} This laboratory also participated in the quality control and assay standardization program in the United States and was 1 of the 6 laboratories that reported RLP-C levels.

Statistical Analysis

All statistical analyses were performed by using SAS version 8.0. Treatment order for this crossover study was assessed by repeated-measures ANOVA models with the use of logarithmic transformations for skewed data. Because some variables were skewed (eg, triglycerides and RLP-C), nonparametric tests were used for these analyses. The 3 statin drugs were further compared with the Friedman test and the Wilcoxon signed rank test for pairwise comparisons. The means of the 3 baselines were compared with the mean of the 2 measurements obtained on therapy or during washout. Spearman correlation coefficients were computed to assess associations between variables of interest. The level of significance was set at \( P<0.05 \) (2-sided test).

Results

The salient characteristics of the participants in the present study were as follows: age 47.2±9.7 years, male/female ratio 10/12, and body mass index 24.5±4.9 kg/m\(^2\).

As is evident in the Table, there was a significant reduction in total cholesterol and LDL-C with all 3 drugs (pravastatin, simvastatin, and atorvastatin). The mean reduction in total cholesterol with pravastatin, simvastatin, and atorvastatin was 16%, 24%, and 27%, respectively (\( P<0.001 \) compared with baseline). The mean reduction in LDL-C with pravastatin, simvastatin, and atorvastatin was 21%, 29%, and 32%, respectively (\( P<0.001 \) compared with baseline). Exclusion of...
Effect of Statin Therapy on Lipid Profile

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol, mg/dL</th>
<th>Triglycerides, mg/dL</th>
<th>LDL-C, mg/dL</th>
<th>HDL-C, mg/dL</th>
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<tr>
<td>Baseline</td>
<td>264±37</td>
<td>230 (173, 280)</td>
<td>170±37</td>
<td>45±13</td>
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<td>Pravastatin</td>
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<td>179 (154, 263)**</td>
<td>133±35*</td>
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</tr>
<tr>
<td>Simvastatin</td>
<td>201±36†</td>
<td>164 (148, 261)*†</td>
<td>118±30†</td>
<td>46±13</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>192±34*†</td>
<td>162 (132, 216)*†</td>
<td>114±28†</td>
<td>45±13</td>
</tr>
<tr>
<td>Mean washout phase</td>
<td>263±32</td>
<td>241 (166, 271)</td>
<td>167±33</td>
<td>47±12</td>
</tr>
</tbody>
</table>

Values are mean±SD for all, except triglycerides, which are expressed as median (25th, 75th percentile).

*P<0.01 vs baseline; †P<0.01 vs pravastatin; and ‡P<0.01 vs atorvastatin.

the patients with triglyceride levels ≥400 mg/dL (n=3) did not change this finding (pravastatin 22%, simvastatin 32%, and atorvastatin 34%, respectively). The percent reduction in total cholesterol and LDL-C with simvastatin and atorvastatin was significantly greater than that with pravastatin (P<0.005). There was a significant reduction in median triglyceride levels with simvastatin (26.3%, P<0.001) and atorvastatin (24.2%, P<0.0001). Pravastatin resulted in a nonsignificant (9.3%) reduction in triglyceride levels (P=0.18). The percent reduction in triglycerides with atorvastin and pravastatin was significantly different (P<0.01).

There were no significant differences in percent reduction in cholesterol, LDL-C, and triglycerides between atorvastatin and simvastatin. None of the drugs had a significant effect on HDL-C levels in these patients, with mean baseline levels of 45±13 mg/dL. According to the new National Cholesterol Education Program (NCEP) guidelines, the secondary goal of treatment is reduction of non-HDL-C. This was also calculated, and the data are shown in Figure 1. Non–HDL-C changes closely mirrored changes in total cholesterol and LDL-C, decreasing significantly by 20.2%, 29.3%, and 31.6% in the pravastatin, simvastatin, and atorvastatin groups (P<0.0001). The reductions in non-HDL-C were not significantly different from the 3 drugs. During the washout periods, lipid and lipoprotein values predictably returned to baseline. Washout periods were not significantly different from each other and, thus, were combined for all parameters. Combined washout data reflected values very similar to the baseline lipid concentrations (Table). Also, in a subset of patients (n=13) in whom there was remaining plasma, apoB levels were measured. There was a significant reduction in apoB levels with all 3 drugs (28%, 37%, and 37%, respectively, with pravastatin, simvastatin, and atorvastatin; P<0.001). No significant differences were seen between the 3 drugs in percent apoB reduction.

Baseline RLP cholesterol values were 13.1±6.8 mg/dL, which was greater than the 90th percentile for men and women in the 5th decade according to recently published population data from the Framingham cohort; the assay was the same assay used in the present study. Statin treatment resulted in variable decreases in RLP-C. Pravastatin therapy did not result in any significant reduction in RLP-C (2.9% reduction, P=0.58 compared with baseline). However, simvastatin and atorvastatin resulted in a significant reduction in median RLP-C levels (for simvastatin, 6.0%, P=0.03; for atorvastatin, 25.9%, P<0.0001) compared with baseline. The reduction of RLP-C with atorvastatin was significantly greater than that with pravastatin (P<0.004) and approached significance (P=0.055) compared with that with simvastatin (Figure 2).

Changes in RLP-C were significantly correlated with decreases in several plasma lipid fractions, including triglycerides (r=0.67, P=0.0006), non–HDL-C (r=0.54, P=0.01), and total cholesterol (r=0.49, P=0.02). There was no signif-

Figure 1. Effect of statin therapy on non-HDL cholesterol levels. Non-HDL cholesterol levels were calculated as the difference between total cholesterol and HDL cholesterol levels. Data are expressed as mean±SD. All 3 statin drug therapies resulted in a significant reduction in non-HDL cholesterol levels compared with baseline (P<0.0001). P indicates pravastatin; S, simvastatin; and A, atorvastatin.

Figure 2. Effect of statin therapy on RLP-C levels. RLP-C levels were assayed as described in Methods. Data are expressed as medians. Simvastatin (S) and atorvastatin (A) resulted in a significant reduction in RLP-C compared with baseline (*P<0.05 for S, and †P<0.001 for A, respectively). Also, atorvastatin resulted in significantly greater reduction in RLP-C compared with pravastatin (P, aP<0.01).
ificant correlation with LDL-C \((r=0.20, P=0.37)\) or HDL-C \((r=0.07, P=0.76)\). The correlation with calculated VLDL cholesterol was not significant \((r=0.35, P=0.12)\). Because we have previously shown in these patients that all 3 statins reduce C-reactive protein levels significantly, we also correlated the change in RLP-C with the change in CRP levels. The correlation was not significant \((r=0.12, P=0.6)\).

Discussion

The efficacy of statin therapy on LDL-C lowering and reduction of cardiovascular events is well established. However, the effect on other atherogenic lipoprotein classes has not been well studied. Increasing evidence points to a significant role for TRLs in atherogenesis. RLPs are products of lipolytic degradation of TRL produced by the liver (VLDL) and intestine (chylomicrons). TRLs are heterogeneous and, therefore, difficult to isolate by use of a single biochemical technique. Recently, a novel method to measure RLPs based on the immunochemical properties of these lipoproteins has become available. This assay has been shown to be a valid measure of atherogenic TRLs. It appears that this RLP-C assay quantifies cholesterol contents of mainly chylomicron remnants as well as a subpopulation of apoE-enriched TRLs. There have been limited studies to date examining the effect of lipid-lowering therapy on RLP-C. The present study is the first study to compare the effects of HMG-CoA reductase inhibitors at equipotent LDL-lowering doses on RLP-C concentrations in patients with combined hyperlipidemia by use of a crossover design. With 22 subjects, the present study has the power of 0.76 at the 0.05 significance level to detect a 15% difference in remnant particle levels between treatments, conservatively assuming a standard deviation of the differences of 25%.

In the present study, all 3 drugs (pravastatin, simvastatin, and atorvastatin) significantly decreased LDL-C. However, only atorvastatin and simvastatin reduced the levels of RLP-C significantly. Pravastatin, although used at a maximum dose (40 mg/d), failed to reduce RLP-C significantly. According to the comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, and fluvastatin in patients with hypercholesterolemia (the CURVES study), in patients with primary hypercholesterolemia, the chosen doses of pravastatin, simvastatin, and atorvastatin decreased LDL by an expected 34%, 35%, and 38%, respectively, and there were no significant differences in LDL-lowering with the 3 drugs at these doses. In the present study, in patients with combined hyperlipidemia, the 3 statins decreased LDL-C slightly less than in the CURVES study (24%, 30%, and 32% for pravastatin, simvastatin, and atorvastatin, respectively). With regard to triglyceride levels, there was a significant reduction in triglycerides with simvastatin and atorvastatin but not with pravastatin therapy. Furthermore, the percent reduction in triglycerides with atorvastatin and pravastatin was significantly different \((P<0.01)\). In the CURVES study, they also reported significant differences in percent reductions in triglyceride levels between atorvastatin (10 mg) therapy versus pravastatin (40 mg) therapy, as seen in the present study. However, most previous trials have been performed with subjects with isolated hypercholesterolemia. One previous study has reported the effect of pravastatin (40 mg/d) in subjects with mixed hyperlipidemia \((n=13\) middle-aged men), as was performed in the present trial, and they also observed only a 20% reduction in LDL and a nonsignificant (4%) reduction in triglycerides. In the large clinical trials with pravastatin, a significant reduction in triglycerides ranging from 11% to 14% compared with placebo was seen; thus, it is possible that the present study did not have a sufficient sample size to confirm this effect. However, it should be pointed out that patients in the present study had higher triglyceride levels than did patients in the large clinical trials and that simvastatin and atorvastatin at lower doses resulted in a significant hypotriglyceridemic effect. Thus, the greater potency of atorvastatin and simvastatin with respect to triglyceride reduction may be more pronounced with a larger sample size. Also, the failure to see a significant effect on HDL-C could be due to the small sample size and the relatively high baseline HDL-C \((45\pm 13 \text{ mg/dL})\) compared with the larger trials (mean levels ranging from 36 to 44 mg/dL). This was also seen in the CURVES study, in which baseline HDL-C levels in patients receiving these doses of statins ranged from 49 to 51 mg/dL and in which no significant effect was seen on HDL-C levels.

With regard to RLP-C levels, our population of patients with combined hyperlipidemia had elevated RLP-C, which exceeded the 90th percentile in North Americans from the Framingham Study cohort. Statin therapy has previously been shown to lower TRL in patients with hypercholesterolemia. Fluvastatin (20 mg/d for 6 weeks) has been shown to significantly decrease levels of IDL \((43\%, P<0.01)\) and apoE \((22\%, P<0.01)\) in patients with heterozygous familial hypercholesterolemia. Two other studies with pravastatin in subjects with moderate hypercholesterolemia have shown similar decreases in IDL \((\approx 45\%)\). With regard to the effect of statin therapy on RLPs, by use of the RLP-C assay, it has recently been shown that high-dose simvastatin \((80 \text{ mg/d for 3 months})\) in 7 patients with familial hypercholesterolemia significantly reduced fasting and postprandial remnant levels, as assessed by the new immunoassay. Although levels in the subjects were very high \((42\pm 19 \text{ mg/dL})\), there was a significant reduction \((13\pm 3 \text{ mg/dL})\) in RLP-C levels with high-dose simvastatin therapy. In the present study, statin therapy (simvastatin and atorvastatin but not pravastatin) produced a significant reduction in RLP-C. This could be explained by 2 factors: (1) baseline RLP-C levels being only double those seen in the normal population, and (2) the dose of statin used. It is possible that greater reductions will be observed with higher doses of statin therapy, and this remains to be established, inasmuch as higher doses result in greater reduction in LDL-C and triglycerides. Recently, Karpe et al have shown in the Lipid Coronary Angiography Trial (LOCAT) that gemfibrozil reduced median RLP-C by 33%. In that trial, there was a very strong correlation between the reduction in RLP-C with reduction in VLDL lipids and plasma triglycerides. Interestingly, our results also suggest a strong correlation between reductions in plasma triglycerides with reductions in RLP-C levels compared with reductions in LDL-C. However, the reduction in triglycerides can only account for \(\approx 44\%\) of the reduction in RLP-C (Pearson correlation on logarithmically transformed data, \(r=0.66)\). Furthermore, although the median reduction in triglycerides with simvastatin and atorvastatin was 26.3% and 24.2%, respectively, the median reduction in RLP was 6% and
25.9%, respectively. If the effects were due solely to an increased clearance of remnants along with LDL via an increased activity of the remnant/LDL receptor, then the percent decreases of LDL-C and RLP-C should be similar. The stronger association with changes in plasma triglycerides with RLP-C suggests that either remnants containing both triglycerides as well as cholesterol were being preferentially cleared compared with LDL or that production rates of VLDL were decreased in addition to enhanced clearance of remnant and LDL particles. Because this study is unique in its crossover design, the differences in statins noted are likely to be due to true differences in drug action compared with differing metabolic environments. Given the relatively small sample size, these conclusions should be treated with caution and confirmed in a larger study using kinetic methodology. Also, in the present study, there was a significant correlation between RLP-C and non–HDL-C in these patients with combined hyperlipidemia. This supports the notion of the ATP III panel, who have suggested the use of non–HDL-C levels in the assessment of atherosclerotic risk in patients with hypertriglyceridemia as a surrogate for RLPs. Two other prospective angiographic trials also suggest that non-LDL apoB-containing lipoproteins or RLPs are more predictive of atherosclerotic progression than are LDL levels.40,41

The effects of statins to reduce circulating RLPs may be due to their effects on lipoprotein kinetics. Also, statins may decrease input rates for VLDL apoB and VLDL triglycerides.7 Studies of the effects of statins on VLDL, IDL, and LDL kinetics in subjects with combined hyperlipidemia have yielded variable results.42–46 However, the exact effect of statins on the uptake of remnants is, as yet, unclear. Although a potential explanation for the disparate results of statin actions in states of hypertriglyceridemia remains elusive, they may simply reflect the heterogeneous nature of metabolic defects leading to combined hyperlipidemia. Inasmuch as cholesterol depletion in the liver with statins could alter VLDL assembly and secretion, it is possible that the reduction in RLP-C seen in the present report is due to decreased production and increased clearance.47 Because atorvastatin appears to be more potent, it is quite possible that in another group of patients, eg, patients with type IV hyperlipidemia, the differences among the statins will be more accentuated.

In summary, in a randomized double-blind crossover trial in subjects with combined hyperlipidemia, we demonstrate for the first time that atorvastatin and simvastatin reduce RLP-C levels significantly, whereas pravastatin does not, and this differential effect is likely mediated by differences in TRL remnant kinetics. Given that RLPs are atherogenic, it is not unreasonable to ascribe part of the benefit of statins in reducing cardiovascular events to the reduction in RLP levels.

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References


