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Relationship of Oxidized Phospholipids and Biomarkers of Oxidized Low-Density Lipoprotein With Cardiovascular Risk Factors, Inflammatory Biomarkers, and Effect of Statin Therapy in Patients With Acute Coronary Syndromes

Results From the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) Trial

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Objectives This study sought to define the relationship between oxidative biomarkers, cardiovascular disease (CVD) risk factors, and inflammatory and thrombosis biomarkers.

Background Elevated levels of oxidized phospholipids (OxPL) on apolipoprotein B particles (apoB) represent a novel biomarker of CVD. Previous studies suggest that an increase in OxPL/apoB reflects a positive response to statins and a low-fat diet.

Methods This study measured OxPL/apoB, lipoprotein (a) [Lp(a)], and oxidized low-density lipoprotein (OxLDL) biomarkers, consisting of immunoglobulin (Ig)G and IgM autoantibodies to malondialdehyde (MDA)-low-density lipoprotein (LDL) and IgG and IgM apoB-100 immune complexes (IC/apoB), at baseline and after 16 weeks of treatment with atorvastatin 80 mg/day or placebo in 2,342 patients with acute coronary syndromes (ACS) enrolled in the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) trial.

Results At baseline, potentially atheroprotective IgM autoantibodies and IgM IC/apoB were lower in male patients, diabetic patients, and patients >65 years of age. Patients with an LDL level greater than the median (122 mg/dl) had higher levels of OxPL/apoB, Lp(a), and OxLDL biomarkers compared with those who had an LDL level less than the median. Atorvastatin resulted in significantly larger changes in all biomarkers in female patients, patients age <65 years, patients with LDL cholesterol <122 mg/dl, nonsmokers, and nondiabetic patients ($p < 0.0001$ for all). In particular, a significant increase in OxPL/apoB in response to atorvastatin was noted in all 20 subgroups evaluated. Weak or no significant correlations were noted between all OxLDL biomarkers and C-reactive protein, serum amyloid A, tissue plasminogen activator, interleukin-6, intercellular adhesion molecule, vascular cell adhesion molecule, P-selectin, and E-selectin at randomization and 16 weeks.

Conclusions In patients with ACS, baseline levels of oxidative biomarkers varied according to specific CVD risk factors and were largely independent of inflammatory biomarkers. Atorvastatin uniformly increased OxPL/apoB levels in all subgroups studied. Future studies are warranted to assess whether the increase in OxPL/apoB levels reflects the benefit of effective therapeutic interventions and prediction of new CVD events. (J Am Coll Cardiol 2009;53: 2186–96) © 2009 by the American College of Cardiology Foundation

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The MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study established that patients presenting with acute coronary syndromes (ACS) treated with atorvastatin 80 mg had a reduction in recurrent ischemic cardiovascular events over a 16-week period compared with placebo (1,2). A previous analysis of the MIRACL trial (3) showed lower apolipoprotein B-100 (apoB) levels in patients treated with atorvastatin than with placebo, but that the remaining apoB particles in atorvastatin-treated patients were more enriched in oxidized phospholipids (OxPL), as measured by antibody E06. Lipoprotein (a) [Lp(a)] binds and transports E06-detectable OxPL found on lipoproteins in human plasma (4–8). Accordingly, atorvastatin treatment in the MIRACL trial resulted in an increase in Lp(a) levels paralleling the increase in OxPL/apoB and reduction in clinical events. In normal subjects as well as in clinically stable patients with dyslipidemia, an increase in OxPL/apoB levels also occurred in response to low-fat diets (9) or statin therapy (10–12). A similar increase has been seen in rabbits and cynomolgus monkeys with pre-existing atherosclerosis, which were subsequently placed on low-fat diets (13). Based on these observations, we previously hypothesized that an increase in OxPL/apoB levels after therapeutic interventions may be a biomarker of plaque stabilization and early atherosclerosis regression.

Although many epidemiological studies show broad associations between oxidized low-density lipoprotein (OxLDL) biomarkers and cardiovascular disease (CVD), little information exists regarding the interaction of clinical characteristics, inflammatory biomarkers, and statin therapy on indicators of OxLDL (14–17). In a previous analysis of the MIRACL trial, we evaluated the overall effect of high-dose atorvastatin in patients with ACS and showed unique changes in a variety of oxidative biomarkers in response to atorvastatin (1,2). In particular, we noted significant increases in OxPL/apoB that mirrored the effect of atorvastatin. In the current analysis, we evaluated the interrelationships between a comprehensive panel of oxidative biomarkers and clinical and demographic descriptors, lipid parameters, and inflammatory biomarkers to assess the impact of atorvastatin therapy in specific and large subgroups of patients. This investigation aimed to gain insights into the potential mechanisms of the early benefit of statin therapy.

Abbreviations and Acronyms

ACS = acute coronary syndrome
apoB = apolipoprotein B-100
CI = confidence interval
CVD = cardiovascular disease
IC/apoB = apolipoprotein B-immune complexes
Ig = immunoglobulin
Lp(a) = lipoprotein (a)
MDA = malondialdehyde
OxLDL = oxidized low-density lipoprotein
OxPL = oxidized phospholipids

Methods

Study design and patient sample. The MIRACL trial enrolled 3,086 patients with unstable angina or non-Q-wave acute myocardial infarction between 24 and 96 h after hospital admission at 122 centers in 19 countries. Patients were randomly assigned to double-blind treatment with atorvastatin 80 mg/day or placebo for 16 weeks. The primary efficacy measure was the time to first occurrence of death, nonfatal acute myocardial infarction, cardiac arrest with resuscitation, or worsening angina with new objective evidence of ischemia and requiring emergency re-hospitalization (1). Blood samples for biomarker analysis were collected at baseline and at 16 weeks of randomized treatment. Blood was collected in ethylenediaminetetraacetic acid and stored at −70°C. Of 2,739 patients who survived to complete the 16-week trial, 2,342 had baseline and week-16 blood samples available and served as the cohort for the present analysis.

Seven hundred forty-four participants in the trial were not included in this analysis because of death before 16-week follow-up or missing laboratory samples. Of the 2,342 patients included, 10.6% had a nonfatal primary end point in the atorvastatin group and 12.2% in the placebo group during the 16-week follow-up period. This corresponds to a 13% relative risk reduction with atorvastatin. In the entire MIRACL study population, fatal and nonfatal primary end point events occurred in 14.8% and 17.6% of the atorvastatin and placebo groups, respectively, corresponding to a 16% relative risk reduction with atorvastatin. The incidence of recurrent events and the relative risk reduction with atorvastatin were lower in the cohort of the present analysis than the entire MIRACL study population because patients who died in the follow-up period did not have a 16-week blood sample and therefore are not represented in this analysis.

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Determination of OxPL/apoB, Lp(a), and apoB levels. Levels of OxPL/apoB, Lp(a), and apoB were measured as previously described (3). The OxPL/apoB measure reflects the content of OxPL on apoB particles, which primarily consist of OxPL detectable with murine monoclonal antibody E06 on Lp(a), where Lp(a) is not necessarily oxidized but acts as carrier of such OxPL. Therefore, OxPL/apoB is not necessarily a biomarker of OxLDL per se, but reflects the biological capacity of Lp(a) to bind and transport OxPL.

Determination of OxLDL biomarkers: immunoglobulin (Ig)G and IgM autoantibodies to malondialdehyde (MDA)-LDL and IgG and IgM ApoB immune complexes (IC/apoB). IgG and IgM autoantibodies to MDA-LDL and IgG and IgM IC/apoB were measured as previously described (3).

Determination of inflammatory biomarker levels. High-sensitivity C-reactive protein, serum amyloid A, and interleukin-6 were measured as previously described (18). Tissue plasminogen activator, intercellular adhesion molecule, vascular cell adhesion molecule, P-selectin, and E-selectin were measured by enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, Minnesota).

Statistical analysis. Because the baseline and week 16 distributions of the OxLDL markers were positively skewed, log-transformed values were used in the statistical models and analyses and antilog-transformed for descriptive statistics yielding geometric means and 95% confidence intervals (CIs) for baseline, week 16, and percent change from baseline to week 16. Inferential analyses included paired-sample *t* tests for within-treatment group differences in markers at baseline versus week 16, and independent-sample *t* tests for between-treatment group differences in markers at week 16 and between-treatment group differences in absolute change from baseline to week 16. All paired- and independent-sample *t* tests were based on log-transformed makers. Spearman correlations (based on the raw data) were calculated to summarize the relationship between OxLDL, lipid, and inflammatory biomarkers. Statistical significance was defined as $p < 0.001$ to account for multiple comparisons. All data management and statistical analyses were carried out under the supervision of Pfizer, Inc.

Results

Patient characteristics. Table 1 shows the baseline characteristics of the study population. The study cohort consisted of 1,549 male patients and 793 female patients, 1,114 patients <65 years of age and 1,228 patients ≥65 years of age, 1,612 patients with hypertension, 526 patients with diabetes mellitus, and 666 current smokers. The presenting symptom was unstable angina in 1,061 patients and non–Q-wave myocardial infarction in 1,281 patients. There were no statistical differences in baseline characteristics between the atorvastatin and placebo groups.

Table 1 Baseline Characteristics of the 2,342 Study Subjects

Characteristic	Placebo (n = 1,191)	Atorvastatin (n = 1,151)
Age, mean (SD), yrs	64 (11)	65 (11)
Men, n (%)	797 (67)	752 (65)
Presenting syndrome, n (%)		
Unstable angina	534 (45)	527 (46)
Non–Q-wave myocardial infarction	657 (55)	624 (54)
Past myocardial infarction, n (%)	288 (24)	269 (23)
Prior coronary revascularization, n (%)	131 (11)	109 (9)
Hyperlipidemia, n (%)	408 (34)	421 (35)
Hypertension, n (%)	636 (53)	629 (55)
Diabetes mellitus type II, n (%)	280 (24)	251 (22)
Total cholesterol, mean (SD), mg/dl	207 (37)	206 (38)
LDL cholesterol, mean (SD), mg/dl	124 (33)	124 (34)
HDL cholesterol, mean (SD), mg/dl	46 (12)	47 (12)
Triglycerides, mean (SD), mg/dl	185 (90)	182 (86)

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Relationship of baseline levels of OxPL/apoB, Lp(a), and OxLDL biomarkers to various cardiovascular risk factors according to median levels of lipid parameters. Table 2 shows the mean (95% CI) levels of the various parameters measured according to specific subgroups of patients and according to less than or greater than median levels of lipid parameters. The OxPL/apoB and Lp(a) levels were highest in patients age <65 years and nondiabetic patients. In general, IgM autoantibodies and ICs tended to be higher in traditionally lower-risk subgroups and IgG autoantibodies and ICs higher in subgroups traditionally at higher risk. All oxidative variables tended to be higher in patients with LDL greater than median (>122 mg/dl) and apoB greater than median (>131 mg/dl).

Changes in OxPL/apoB and Lp(a) according to subgroups in response to atorvastatin versus placebo. In a previous analysis (3), we reported that atorvastatin, compared with placebo, resulted in significant increases in OxPL/apoB (9.5% vs. −3.9%, $p < 0.0001$) and Lp(a) (8.8% vs. −0.7%, $p < 0.0001$) over the 16-week treatment period. Furthermore, IgM IC/apoB increased in the atorvastatin group but decreased in the placebo group at 16 weeks (15.4% vs. −9.3%, $p < 0.0001$) and were associated with reduced risk of subsequent events.

In the current analysis, we evaluate changes in these OxLDL biomarkers in specific subgroups of patients. Figure 1 shows treatment effects of atorvastatin versus placebo in specific subgroups, as well as within-subgroup differences in OxPL/apoB and Lp(a) in both the placebo and the atorvastatin groups individually. There was a consistently and significantly higher increase in OxPL/apoB in all 12 subgroups of cardiovascular risk factors and across all 8 subgroups of lipoproteins dichotomized by median levels in response to

Table 2 Baseline Levels of OxLDL Biomarkers [Mean (95% CI)] in Patients With Various Cardiovascular Risk Factors and According to Median Lipid Profile Values on Presentation

Variable	OxPL/apoB	Lp(a)	MDA-LDL IgG	MDA-LDL IgM	IC/apoB IgG	IC/apoB IgM
Sex						
Male	10,125 (9,845–10,412)	11.7 (11.0–12.3)	4,519 (4,401–4,641)	9,437 (9,168–9,714)	8,397 (8,167–8,632)	3,410 (3,290–3,535)
Female	10,013 (9,632–10,410)	12.7 (11.7–13.8)	4,303 (4,119–4,494)	11,446 (11,002–11,909)	8,128 (7,801–8,468)	3,582 (3,410–3,763)
p Value	0.65	0.08	0.059	**<0.0001**	0.18	0.12
Age, yrs						
<65	10,365 (10,022–10,721)	12.5 (11.7–13.3)	4,352 (4,202–4,506)	10,328 (9,994–10,673)	8,299 (8,029–8,579)	3,888 (3,741–4,041)
≥65	9,841 (9,545–10,146)	11.6 (10.9–12.4)	4,531 (4,396–4,670)	9,850 (9,521–10,189)	8,310 (8,049–8,579)	3,125 (2,997–3,260)
p Value	0.025	0.13	0.086	0.049	0.96	**<0.0001**
Hypertension						
Yes	10,060 (9,790–10,338)	11.8 (11.2–12.5)	4,475 (4,350–4,605)	10,117 (9,831–10,410)	8,510 (8,274–8,752)	3,443 (3,321–3,568)
No	10,062 (9,658–10,483)	12.2 (11.2–13.3)	4,392 (4,223–4,567)	9,991 (9,572–10,428)	7,911 (7,600–8,234)	3,536 (3,366–3,714)
p Value	0.99	0.55	0.44	0.63	0.004	0.39
Diabetes mellitus						
Yes	9,571 (9,143–10,019)	10.9 (9.9–11.9)	4,480 (4,268–4,702)	9,511 (9,037–10,010)	8,626 (8,207–9,066)	3,382 (3,181–3,595)
No	10,208 (9,946–10,478)	12.3 (11.7–13.0)	4,441 (4,326–4,559)	10,250 (9,979–10,529)	8,234 (8,023–8,451)	3,497 (3,384–3,615)
p Value	0.019	0.029	0.76	**0.001**	0.099	0.34
Active smoking						
Yes	10,207 (9,775–10,658)	12.3 (11.3–13.4)	4,558 (4,370–4,755)	10,066 (9,622–10,530)	8,301 (7,936–8,683)	3,555 (3,372–3,749)
No	10,003 (9,740–10,272)	11.8 (11.2–12.5)	4,407 (4,287–4,530)	10,082 (9,805–10,369)	8,330 (8,109–8,556)	3,437 (3,320–3,559)
p Value	0.43	0.46	0.19	0.95	0.89	0.30
Inclusion event						
Non–Q-wave MI	9,965 (9,663–10,277)	12.0 (11.2–12.7)	4,232 (4,098–4,371)	9,870 (9,552–10,198)	7,864 (7,633–8,103)	3,404 (3,273–3,541)
Unstable angina	10,236 (9,898–10,585)	12.0 (11.3–12.9)	4,716 (4,566–4,870)	10,327 (9,980–10,686)	8,869 (8,562–9,187)	3,545 (3,397–3,700)
p Value	0.25	0.86	**<0.0001**	0.06	**<0.0001**	0.17
Apo B-100, mg/dl						
<131	9,998 (9,685–10,320)	11.8 (11.1–12.6)	4,296 (4,153–4,444)	9,815 (9,492–10,149)	7,947 (7,690–8,212)	3,389 (3,252–3,532)
≥131	10,176 (9,849–10,515)	12.3 (11.5–13.1)	4,592 (4,452–4,737)	10,341 (9,998–10,696)	8,703 (8,428–8,988)	3,551 (3,408–3,699)
p Value	0.45	0.44	0.004	0.031	**0.0001**	0.12
Total cholesterol, mg/dl						
<206	9,914 (9,601–10,237)	11.6 (10.9–12.4)	4,332 (4,200–4,468)	9,752 (9,424–10,092)	8,141 (7,873–8,418)	3,430 (3,291–3,575)
≥206	10,208 (9,886–10,541)	12.3 (11.5–13.1)	4,569 (4,416–4,728)	10,411 (10,073–10,760)	8,504 (8,239–8,778)	3,512 (3,371–3,659)
p Value	0.21	0.24	0.023	0.97	0.06	0.42
LDL cholesterol, mg/dl						
<122	9,655 (9,356–9,963)	10.8 (10.2–11.6)	4,209 (4,066–4,357)	9,816 (9,488–10,156)	7,781 (7,529–8,041)	3,360 (3,224–3,503)
≥122	10,507 (10,165–10,860)	13.3 (12.5–14.2)	4,711 (4,568–4,858)	10,348 (10,004–10,704)	8,901 (8,619–9,194)	3,584 (3,438–3,736)
p Value	**0.0003**	**<0.0001**	**<0.0001**	0.031	**<0.0001**	0.031
HDL cholesterol, mg/dl						
<45	9,700 (9,398–10,012)	10.7 (10.0–11.4)	4,410 (4,273–4,550)	9,914 (9,582–10,258)	8,494 (8,217–8,780)	3,565 (3,426–3,708)
≥45	10,416 (10,085–10,759)	13.3 (12.5–14.2)	4,493 (4,344–4,646)	10,241 (9,907–10,587)	8,164 (7,906–8,430)	3,388 (3,247–3,535)
p Value	0.002	**<0.0001**	0.43	0.18	0.092	0.086
Triglycerides, mg/dl						
<164	10,633 (10,283–10,996)	13.8 (12.9–14.7)	4,583 (4,427–4,744)	10,082 (9,741–10,436)	8,875 (8,581–9,179)	3,470 (3,324–3,622)
≥164	9,533 (9,248–9,826)	10.4 (9.8–11.1)	4,324 (4,194–4,458)	10,074 (9,748–10,411)	7,815 (7,576–8,062)	3,472 (3,337–3,611)
p Value	**<0.0001**	**<0.0001**	0.014	0.97	**<0.0001**	0.99

For OxPL/apoB, IgG, and IgM, IgG IC/apoB and autoantibodies to MDA-LDL, units are in relative light units. Lp(a) units are mg/dl. The p values in **bold** represent significant changes defined as $p < 0.001$.

apoB = apolipoprotein B-100; CI = confidence interval; HDL = high-density lipoprotein; IC/apoB = apolipoprotein B-immune complexes; Ig = immunoglobulin; LDL = low-density lipoprotein; Lp(a) = lipoprotein (a); MDA = malondialdehyde; MI = myocardial infarction; OxLDL = oxidized low-density lipoprotein; OxPL = oxidized phospholipids; OxPL/apoB = oxidized phospholipids as measured by antibody E06.

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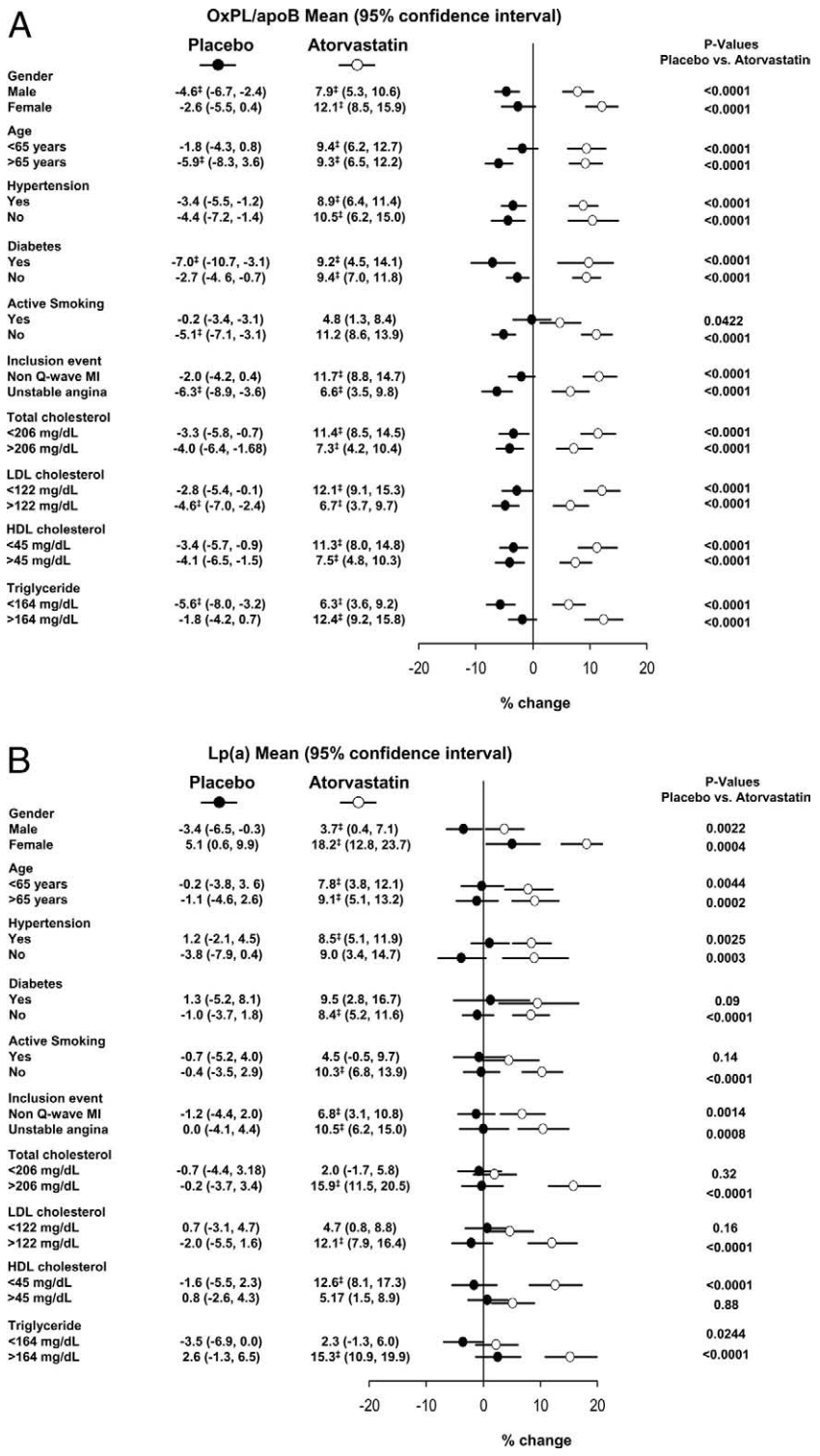

Figure 1 **Change in OxPL/apoB and Lp(a) Levels in Various Subgroups in Response to Atorvastatin**

The panels represent the mean percent change (95% confidence interval) from baseline to week 16 in OxPL/apoB **(A)** and Lp(a) **(B)** levels in the various subgroups according to treatment with atorvastatin versus placebo. The p values listed in the **right margin** in each panel represent differences between placebo versus atorvastatin in the various subgroups. ‡Within-group mean percent changes from baseline to week 16 in the placebo or atorvastatin groups individually (‡$p < 0.001$). HDL = high-density lipoprotein; Lp(a) = lipoprotein(a); LDL = low-density lipoprotein; MI = myocardial infarction; OxPL/apoB = oxidized phospholipids as measured by antibody E06.

atorvastatin therapy compared with placebo (Fig. 1A). Changes in Lp(a) were similar to changes in OxPL/apoB, except that the changes were not as uniform as seen with OxPL/apoB (Fig. 1B).

Evaluating within-group changes (p values for within-group changes in Figure 1 are represented by the symbol ‡ next to the numerical data) in the atorvastatin group alone, increases in OxPL/apoB (ranging from 4.8% to 12.4%) from baseline to 16 weeks were noted in all subgroups (Fig. 1A). Similarly, in response to atorvastatin, Lp(a) increased in all subgroups (range 3.7% to 18.1%), except in male patients, current smokers, those with triglyceride levels less than the median, and those with total cholesterol levels greater than the median (Fig. 1B). In contrast, within-group changes in the placebo-treated group showed mainly reductions or no changes in OxPL/apoB or Lp(a) over the 16-week observation period.

Changes in autoantibodies to MDA-LDL and ICs according to subgroups in response to atorvastatin versus placebo. Over the 16-week treatment period, IgG IC/apoB increased in all subgroups of the atorvastatin group (range 3% to 20%), but in none of the subgroups of the placebo group. For IgM IC/apoB, significant increases were noted in subgroups of both the placebo and atorvastatin groups. For both IgG and IgM MDA-LDL autoantibody levels, highly significant increases (approximately 5% to 15%, $p < 0.001$ for all) were noted in all subgroups of both treatment groups. As opposed to subgroup analysis for OxPL/apoB and Lp(a), homogeneity was not present among subgroups of each treatment group in these responses (Table 3).

Correlations among OxLDL biomarkers at randomization. Table 4 shows the correlations between OxPL/apoB, Lp(a), and OxLDL biomarkers at the baseline time point at randomization. The OxPL/apoB levels correlated strongly with Lp(a) ($r = 0.68$, $p < 0.0001$) and weakly, but significantly, with all other indirect OxLDL measures. All IgG and IgM OxLDL biomarkers correlated with each other modestly. Similar correlations were noted at the end of the 16-week study period (data not shown).

Correlations between OxLDL biomarkers and inflammatory biomarkers at randomization. Table 5 shows the correlation analysis between OxPL/apoB, Lp(a), OxLDL biomarkers, lipid parameters, and inflammatory and thrombosis biomarkers. Modest to weak, but statistically significant, correlations were noted between total cholesterol and LDL cholesterol (LDL-C) and essentially all oxidative biomarkers. Significant positive and negative correlations were noted between several of the oxidative biomarkers and inflammatory markers, but most of these were quite weak. Of interest, OxPL/apoB and Lp(a) did not correlate with high-sensitivity C-reactive protein, serum amyloid A, or interleukin-6. Overall similar findings were noted at the end of the 16-week study period (data not shown).

Discussion

The most remarkable finding of this analysis of the MIRACL study, encompassing a large cohort of patients with ACS in a placebo-controlled trial, was that the increase in OxPL/apoB levels in response to atorvastatin was consistently and significantly increased in all 12 subgroups of CVD risk factors and across all 8 subgroups of lipoproteins. Based on previous studies in experimental animal models, and in observations in smaller cohorts of patients on diets or statins, we previously suggested that an increase in OxPL/apoB levels in response to intervention may reflect plaque regression and/or stabilization (13,19,20). These results strongly argue that future studies are warranted to assess whether the increase in OxPL/apoB will be a biomarker of an effective response to therapeutic interventions and prediction of new events, either in the setting of ACS or in more stable patients over a longer time interval.

Relationship of OxPL/apoB levels to statin therapy and low-fat diets. The effect of therapeutic interventions on biomarkers of OxLDL in humans is now undergoing detailed evaluation. The increase in OxPL/apoB in response to atorvastatin was initially noted in the MIRACL study (3). Subsequently, several additional studies have validated this observation using various doses of pravastatin (10–12) or atorvastatin (11,12) and also in young women (9) or children with familial hypercholesterolemia consuming low-fat diets (10), showing a 9% to 48% increases in OxPL/apoB. In cynomolgus monkeys and New Zealand White rabbits with established atherosclerosis, 50% to 100% increases in OxPL/apoB were noted following regression diets. These increases were associated with a near-complete depletion of OxPL and malondialdehyde epitopes and other measures of oxidative stress in aortic atherosclerotic lesions, which coincided with concomitant evidence of plaque stabilization as indicated by loss of plaque macrophages and increased smooth muscle cell and collagen content (13,19,20). Similar observations showing reduced oxidative stress and inflammation with concomitant plaque stabilization were noted in human carotid endarterectomy plaques after 3 months of treatment with pravastatin (21). Therefore, this evidence supporting the utility of oxidation-specific epitopes to monitor biologically relevant changes in atherosclerotic lesions and in plasma after therapeutic interventions provides a strong rationale for further study. It bears emphasis that the OxPL/apoB measure only detects OxPL on apoB particles, and primarily on Lp(a), and that it does not detect OxPL in plasma or OxPL not recognized by antibody E06 (22).

In the current study, OxPL/apoB and Lp(a) did not change significantly in the placebo group or any placebo subgroups. Overall in the MIRACL trial, reduction of cardiovascular events was not related to any significant interaction of treatment assignment with age, gender, diabetes, or current smoking, although the power of the study may limit the conclusions of such analyses. We have previously shown increases in OxPL/apoB and Lp(a) as

Table 3 Within-Group Changes in the Mean Percent Change From Baseline in Immune Complexes and Autoantibodies to MDA-LDL [Mean (95% CI)]

	IC/apoB IgG		IC/apoB IgM		MDA-LDL IgG		MDA-LDL IgM	
Variable	Placebo	Atorvastatin	Placebo	Atorvastatin	Placebo	Atorvastatin	Placebo	Atorvastatin
Sex								
Male	−1.1 (−4.6 to 2.4)	8.7* (5.0 to 12.6)	11.1* (7.5 to 14.7)	15.6* (11.8 to 19.4)	10.9* (8.5 to 13.4)	12.4* (9.9 to 14.9)	12.4* (10.1 to 14.6)	8.8* (6.5 to 11.2)
Female	−3.0 (−7.6 to 1.8)	11.4* (6.1 to 17.0)	5.8 (0.2 to 11.7)	15.6* (10.7 to 20.7)	10.6* (7.2 to 14.3)	18.4* (12.5 to 24.7)	7.9* (5.3 to 10.6)	9.4* (6.3 to 12.6)
Age, yrs								
<65	−0.1 (−4.0 to 3.9)	9.7* (5.0 to 14.6)	11.7* (7.9 to 15.7)	14.4* (10.2 to 18.7)	12.2* (9.4 to 15.2)	12.5* (8.1 to 17.1)	13.1* (10.7 to 15.6)	8.1* (5.4 to 10.7)
≥65	−3.3 (−7.1 to 0.7)	9.7* (5.6 to 13.9)	7.0 (2.4 to 11.7)	16.7* (12.5 to 21.0)	9.5* (6.7 to 12.4)	16.1* (13.2 to 19.1)	8.7* (6.3 to 11.2)	9.8* (7.2 to 12.5)
Hypertension								
Yes	−3.4 (−6.7 to −0.0)	9.1* (5.5 to 12.7)	8.6* (4.7 to 12.6)	14.7* (11.1 to 18.5)	10.1* (7.7 to 12.6)	13.9* (10.6 to 17.3)	10.5* (8.5 to 12.6)	9.2* (6.9 to 11.6)
No	2.0 (−3.0 to 7.2)	10.8* (4.6 to 17.1)	11.1* (6.5 to 15.9)	17.7* (12.6 to 23.1)	12.8* (9.1 to 16.5)	16.0* (12.1 to 20.0)	11.7* (8.5 to 15.1)	8.8* (5.8 to 11.9)
Diabetes								
Yes	−5.3 (−10.8 to 0.5)	16.6* (9.9 to 23.8)	8.3 (2.2 to 14.8)	21.2* (14.3 to 28.6)	9.6* (5.3 to 14.1)	13.5* (8.7 to 18.5)	11.7* (8.1 to 15.5)	10.5* (6.3 to 14.8)
No	−0.6 (−3.8 to 2.7)	7.7* (4.2 to 11.3)	9.7* (6.3 to 13.3)	14.1* (10.8 to 17.5)	11.4* (9.1 to 13.7)	14.8* (11.7 to 17.9)	10.7* (8.7 to 12.7)	8.7* (6.6 to 10.8)
Active smoking								
Yes	4.4 (−0.9 to 9.9)	8.7 (3.1 to 14.6)	14.3* (9.0 to 19.8)	17.8* (11.9 to 24.1)	15.4* (11.3 to 19.6)	14.9* (11.1 to 18.8)	17.1* (13.5 to 20.9)	14.9* (11.0 to 19.0)
No	−4.2 (−7.4 to −0.8)	9.9* (6.2 to 13.7)	7.5* (3.8 to 11.3)	14.7* (11.3 to 18.2)	9.2* (6.9 to 11.5)	14.4* (11.0 to 17.8)	8.5* (6.5 to 10.4)	6.9* (4.8 to 9.0)
Inclusion event								
Non–Q-wave MI	2.4 (−1.4 to 6.3)	12.6* (8.4 to 16.9)	15.5* (11.5 to 19.6)	18.0* (13.9 to 22.2)	16.3* (13.5 to 19.2)	17.9* (13.8 to 22.1)	13.8* (11.4 to 16.2)	10.2* (7.6 to 12.8)
Unstable angina	−6.6 (−10.5 to −2.5)	6.3 (1.8 to 11.0)	2.2 (−2.3 to 6.8)	12.9* (8.6 to 17.3)	4.4* (1.64 to 7.3)	10.5* (7.5 to 13.7)	7.4* (4.9 to 9.9)	7.7* (5.0 to 10.4)
Total cholesterol, mg/dl								
<206	0.9 (−2.9 to 4.9)	16.4* (11.7 to 21.2)	13.0* (8.8 to 17.4)	18.8* (14.4 to 235)	15.4* (12.4 to 18.4)	18.6* (15.6 to 21.6)	13.4* (10.7 to 16.2)	10.9* (8.1 to 13.8)
≥206	−4.3 (−8.1 to −0.2)	3.0 (−1.0 to 7.19)	6.1 (1.7 to 10.6)	12.4* (8.4 to 16.4)	6.9* (4.1 to 9.6)	10.5* (6.3 to 14.8)	8.6* (6.4 to 10.8)	7.2* (4.9 to 9.7)
LDL cholesterol, mg/dl								
<122	2.4 (−1.6 to 6.6)	16.0* (11.4 to 20.7)	13.5* (8.7 to 18.6)	19.5* (15.2 to 24.0)	16.9* (13.9 to 19.9)	19.8* (15.4 to 24.5)	13.5* (10.8 to 16.2)	10.3* (7.7 to 13.1)
≥122	−5.8 (−9.6 to −1.8)	3.16 (−0.9 to 7.4)	5.6 (1.8 to 9.5)	11.7* (7.6 to 16.0)	5.7* (3.0 to 8.5)	9.1* (6.2 to 12.0)	8.7* (6.39 to 11.0)	8.0* (5.4 to 10.7)
HDL cholesterol, mg/dl								
<45	1.3 (−2.7 to 5.4)	9.2* (4.7 to 13.8)	10.3* (6.2 to 14.5)	13.7* (9.6 to 17.9)	11.6* (8.6 to 14.6)	12.8* (9.8 to 15.9)	11.4* (8.8 to 14.1)	7.9* (5.2 to 10.8)
≥45	−4.7 (−8.5 to −0.7)	9.8* (5.6 to 14.1)	8.5* (4.1 to 13.2)	17.2* (12.9 to 21.6)	10.4* (7.6 to 13.2)	15.9* (11.7 to 20.2)	10.4* (8.1 to 12.8)	10.1* (7.6 to 12.7)
Triglycerides, mg/dl								
<164	−6.6 (−10.4 to −2.6)	6.4 (0.2 to 10.7)	5.8* (1.4 to 10.4)	14.9* (10.9 to 19.0)	9.5* (6.9 to 12.3)	16.2* (12.0 to 20.6)	11.0* (8.5 to 13.5)	10.7* (8.3 to 13.3)
≥164	3.1 (−0.9 to 7.2)	12.8* (8.3 to 17.5)	12.9 (8.8 to 17.2)	16.3* (11.9 to 20.9)	12.3* (9.3 to 15.4)	12.8* (9.8 to 16.0)	10.8* (8.38 to 13.3)	7.5* (4.7 to 10.3)

*$p < 0.001$ for within-group comparison of baseline and 16-week levels.
MI = myocardial infarction; other abbreviations as in Table 1.

Table 4 Correlations Among OxLDL Biomarkers at Randomization

	OxPL/apoB	Lp(a)	IC/apoB IgG	IC/apoB IgM	MDA-LDL IgG	MDA-LDL IgM
OxPL/apoB	—	r = 0.68 p < 0.0001	r = 0.15 p < 0.0001	r = 0.15 p < 0.0001	r = 0.09 p < 0.0001	r = 0.05 p = 0.019
Lp(a)	—	—	r = −0.08 p = 0.0002	r = 0.09 p < 0.0001	r = 0.01 p = 0.75	r = 0.05 p = 0.009
IC/apoB IgG	—	—	—	r = 0.31 p < 0.0001	r = 0.47 p < 0.0001	r = 0.11 p < 0.0001
IC/apoB IgM	—	—	—	—	r = 0.20 p < 0.0001	r = 0.65 p < 0.0001
MDA-LDL IgG	—	—	—	—	—	r = 0.19 p < 0.0001
MDA-LDL IgM	—	—	—	—	—	—

Values are Spearman rank correlations: coefficient: r, p value.
Abbreviations as in Table 1.

acute phase reactants after myocardial infarction that can remain elevated for 6 months (6). Unfortunately, the current data do not allow us to test whether increases in OxPL/apoB or Lp(a) in the atorvastatin group relate to decreased cardiovascular risk, because one cannot use values of biomarkers at week 16 or changes in biomarkers between baseline and week 16 to determine a relation to events before week 16. To validate the hypothesis that an increase in OxPL/apoB can serve as a biomarker of efficacious therapy, future studies in patients would need to determine a change in OxPL/apoB in response to therapeutic interventions, follow up subjects for events, and assess whether the change in OxPL/apoB was a predictor of the observed benefit. Alternatively one may document loss of oxidation-specific epitopes in the vessel wall, as has been recently documented in mice using magnetic resonance techniques (23), in conjunction with an increase in plasma levels of OxPL/apoB. In contrast to studies in ACS in which these biomarkers may be affected by the severity of the underlying presentation, studies in stable subjects without pre-existing CVD have shown a strong predictive value of OxPL/apoB and Lp(a) measures (24,25).

Relationship of OxPL/apoB, Lp(a), and OxLDL biomarkers to demographic and lipid variables. This analysis of the MIRACL study further shows significant baseline differences in levels of OxPL/apoB, Lp(a), and OxLDL biomarkers among a variety of subgroups in patients presenting with ACS. For example, IgM autoantibodies and

Table 5 Correlation Between OxLDL Biomarkers With Lipid Parameters and Inflammatory Markers at Randomization

	OxPL/apoB	Lp (a)	MDA-LDL IgG	MDA-LDL IgM	IC/apoB IgG	IC/apoB IgM
ApoB-100	r = 0.01 p = 0.80	r = 0.01 p = 0.73	r = 0.08 p < 0.0001	r = 0.06 p = 0.007	r = 0.12 p < 0.0001	r = 0.05 p = 0.011
LDL cholesterol	r = 0.08 p = 0.0002	r = 0.11 p < 0.0001	r = 0.10 p < 0.0001	r = 0.06 p = 0.007	r = 0.14 p < 0.0001	r = 0.07 p = 0.001
HDL cholesterol	r = 0.06 p = 0.004	r = 0.10 p < 0.0001	r = −0.00 p = 0.91	r = 0.03 p = 0.19	r = −0.05 p = 0.030	r = −0.04 p = 0.08
Triglycerides	r = −0.12 p < 0.0001	r = −0.16 p < 0.0001	r = −0.061 p = 0.003	r = 0.02 p = 0.45	r = −0.12 p < 0.0001	r = −0.01 p = 0.73
Total cholesterol	r = 0.04 p = 0.08	r = 0.05 p = 0.027	r = 0.06 p = 0.006	r = 0.07 p = 0.001	r = 0.05 p = 0.017	r = 0.04 p = 0.053
hs-CRP	r = −0.02 p = 0.39	r = 0.03 p = 0.10	r = −0.06 p = 0.003	r = −0.05 p = 0.015	r = −0.01 p = 0.80	r = −0.06 p = 0.007
SAA	r = −0.04 p = 0.07	r = 0.02 p = 0.27	r = −0.08 p = 0.0002	r = −0.06 p = 0.007	r = −0.02 p = 0.36	r = −0.07 p = 0.0006
IL-6	r = −0.03 p = 0.16	r = 0.01 p = 0.49	r = 0.04 p = 0.07	r = −0.04 p = 0.07	r = 0.07 p = 0.002	r = −0.06 p = 0.006
t-PA	r = −0.01 r = 0.74	r = −0.06 p = 0.008	r = 0.03 p = 0.20	r = 0.02 p = 0.42	r = −0.06 p = 0.006	r = −0.02 p = 0.33
ICAM	r = −0.08 p < 0.0001	r = −0.07 p = 0.002	r = 0.03 p = 0.13	r = 0.09 p < 0.0001	r = −0.01 p = 0.93	r = 0.04 p = 0.035
VCAM	r = −0.06 p = 0.002	r = −0.04 p = 0.06	r = 0.12 p < 0.0001	r = 0.06 p = 0.006	r = 0.02 p = 0.42	r = 0.02 p = 0.24
P-selectin	r = −0.02 p = 0.48	r = −0.02 p = 0.35	r = 0.01 p = 0.64	r = 0.01 p = 0.64	r = 0.04 p = 0.05	r = 0.03 p = 0.16

Values are Spearman rank correlations: coefficient: r, p value.
Hs-CRP = high-sensitivity C-reactive protein; ICAM = intercellular adhesion molecule; IL = interleukin; SAA = serum amyloid A; t-PA = tissue plasminogen activator, VCAM = vascular cell adhesion molecule; other abbreviations as in Table 1.

IgM IC/apoB, which have been previously suggested to be atheroprotective (3,26,27), were lower in groups traditionally associated with higher CVD risk, such as male patients, diabetic patients, and older patients. In contrast, patients with LDL-C above the median level of 122 mg/dl tended to have higher levels of most oxidative biomarkers. The effect of atorvastatin on OxPL/apoB and OxLDL biomarkers was generally stronger in traditionally lower-risk subgroups, such as female patients, younger patients, nonsmokers, nondiabetic patients, and patients with lower LDL-C.

The OxPL/apoB and Lp(a) levels tended not to be associated with other CVD risk factors. The OxPL/apoB and Lp(a) levels were not correlated with apoB-100, but were higher in patients with LDL-C greater than the median (122 mg/dl), HDL-C >45 mg/dl, and triglycerides <164 mg/dl. It is well appreciated that the OxPL/apoB measure correlates with Lp(a), but this is dependent on the apolipoprotein(a) isoform size as measured by kringle IV repeats (22,28). The Lp(a) levels are genetically determined, and therefore, OxPL/apoB levels tend to reflect this genetic predisposition of Lp(a) levels.

Since the initial description of autoantibodies to OxLDL approximately 20 years ago (29–33), several concepts with consistent scientific evidence have emerged regarding autoantibodies to OxLDL and apoB-immune complexes: 1) They are present in plasma of atherosclerotic animals and patients with CVD. 2) They are also present in normal subjects of all ages, often with high titers. Interestingly, children have been documented to have low levels of IgG autoantibodies to OxLDL, which subsequently increase in young adulthood and older age. In contrast, IgM autoantibodies are higher earlier in life, but tend to decline during older age, when most CVD events occur (34–36); 3) The IgG and IgM autoantibody titers are not always highly correlated, and the same subjects may have high levels of one and low levels of the other. Female patients tend to have higher levels of IgM autoantibodies than male patients. 4) They display an acute phase response in acutely ill patients, often increasing significantly within hours to days after an event. 5) They may have different influences on atherogenesis, at least in experimental models, in which IgMs, which are more likely to be enriched in natural antibodies present even at birth (26) seem to be atheroprotective, but IgGs seem to be atherogenic, except perhaps in response to vaccination, in which they may be associated with atheroprotection (10,26,34,35,37–39). In humans, we have recently shown that IgM OxLDL autoantibodies and IgM apoB-ICs were associated inversely with the presence of angiographically determined CAD, whereas IgG OxLDL autoantibodies and IgG apoB-ICs were positively associated (40). Consistent with prior studies in smaller numbers of subjects, this study showed higher MDA-LDL IgM levels in female patients, patients <65 years of age, and nondiabetic patients, and that younger patients also had higher IC/apoB IgM levels. Additionally, increased baseline levels of IC/apoB IgM were previously shown to predict a reduced risk of recurrent events in the MIRACL trial (3). Future studies evaluating such biomarkers will need to take into account the age and sex of patients for proper comparison and interpretation, particularly in therapeutic studies (17).

The study also showed that both IgG and IgM autoantibodies and IC measures tended to be higher in subjects with elevated apoB-100 or LDL-C levels. This finding is not surprising, because subjects with higher levels of such lipoproteins would be more prone to generate OxLDL in the vessel wall and other sites, which may potentiate an immune response to such oxidation-specific epitopes and augment such titers. Interestingly, there were no differences in these measures when subjects were evaluated by median high-density lipoprotein cholesterol levels.

In prior smaller studies, atorvastatin prevented the expected increase in IgG OxLDL autoantibodies in patients with ACS (41), or led to overall reductions in levels in stable patients (42). Other studies have not suggested an effect of statins on autoantibodies to OxLDL in children with familial hypercholesterolemia or in patients with hypertension (10,43). Another study with rosuvastatin had inconsistent results (44). A limitation in interpreting these studies is the lack of uniform evaluation with equipotent doses of statins in homogeneous populations. In the current study, the expected increase in autoantibodies and ICs was also noted, but there were no significant differences between subgroups or within subgroups in both the placebo and the atorvastatin groups.

OxLDL biomarkers as acute-phase reactants. The MIRACL study represents the largest study in ACS subjects to evaluate the temporal relationship of OxLDL after cardiovascular events. Significant within-group increases in both the placebo and the atorvastatin groups were generally observed in all autoantibody and ICs at the 16-week time point compared with baseline, but changes did not differ among the demographic and lipid-variable subgroups. These findings are consistent with several small studies that have shown a transient elevation in autoantibodies to OxLDL after ACS or percutaneous coronary intervention, which return to baseline over the next few hours, days, or months depending on the severity of the presentation (3,6,41,45,46).

Relationship of oxidative biomarkers to inflammatory and thrombosis biomarkers. In the current analysis, we showed very weak or no correlation between oxidative biomarkers and C-reactive protein, serum amyloid A, tissue plasminogen activator, interleukin-6, intercellular adhesion molecule, vascular cell adhesion molecule, P-selectin, and E-selectin. Although the Spearman correlation coefficient was statistically significant in some cases, this relates more to large sample size than to strong correlation. In most cases, <1% of the variability in inflammatory biomarkers was explained by levels of oxidized lipid markers. However, these oxidative biomarkers correlate modestly to strongly with each other, suggesting that they have a distinct pathophysiological relationship in atherogenesis. In turn,

the lack of correlation of the lipid and oxidized lipid epitopes with C-reactive protein supports the view that C-reactive protein monitors an aspect of risk not reflected by established or novel lipid markers.

Study limitations. Because blood samples for biomarker analysis were not obtained at an intermediate time point during randomized treatment, the current analysis excludes patients who died during the trial or did not provide a 16-week sample for other reasons. This study only included subjects with ACS treated with 1 dose of 1 statin, therefore extrapolation to all subjects with CVD or to treatment with other statins or different doses of atorvastatin should be made with this in mind. Because baseline blood samples were collected up to 4 days after admission to the hospital, and considering that lipoproteins and inflammatory biomarkers are acute-phase reactants, these values may not reflect a true physiologic baseline. This analysis was not prespecified, and therefore the findings should be interpreted as hypothesis generating until confirmed in a prospective analysis.

Conclusions

In the current analysis, we show changes in OxLDL biomarkers in response to statin therapy in large and specific subgroups of patients that have not been documented previously and provide insights into the interrelationships of these biomarkers to clinical, demographic, and inflammatory variables. The consistent increase in OxPL/apoB, and to a lesser extent Lp(a), in response to atorvastatin across all subgroups tested suggests that it may serve as a benchmark for future studies evaluating such biomarkers. Future studies are warranted to assess whether changes in these biomarkers reflect therapeutic efficacy and predict clinical events.

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