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Biomarkers

# Prediction of Cardiovascular Events in Statin-Treated Stable Coronary Patients by Lipid and Nonlipid Biomarkers

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**Objectives** The aim of this study was to investigate the relationship between lipid and nonlipid biomarker levels achieved during statin therapy and the incidence of major cardiovascular events (MCVEs) in patients with stable coronary heart disease (CHD).

**Background** Several plasma nonlipid biomarkers have been shown to predict MCVEs in population studies.

**Methods** This is a nested case-control study in the TNT (Treating to New Targets) study population, a randomized trial that compared the efficacy of high- (80 mg) versus low-dose (10 mg) atorvastatin for the secondary prevention of CHD. Fasting plasma levels of standard lipids and of 18 nonlipid biomarkers were obtained after an 8-week run-in period on atorvastatin 10 mg and again 1 year after being randomized to 10 or 80 mg atorvastatin in 507 patients who experienced MCVEs during the 4.9 years of study follow-up and in 1,020 control subjects. An MCVE was defined as CHD death; nonfatal, non–procedure-related myocardial infarction; resuscitated cardiac arrest; or fatal or nonfatal stroke.

**Results** Low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides were all predictive of recurrent MCVEs ($p \leq 0.009$). Concentrations of many of the 18 nonlipid biomarkers were lowered by atorvastatin therapy (independent of dose). However, almost none of the nonlipid biomarker levels, whether measured after the 8-week run-in period or after 1 year of treatment with 10 or 80 mg atorvastatin, were predictive of recurrent MCVEs.

**Conclusions** In patients with stable CHD, atorvastatin improved plasma levels of an expanded panel of nonlipid biomarkers. However, independently of atorvastatin dose, the achieved levels of the vast majority of nonlipid biomarkers did not predict MCVEs. (A Study to Determine the Degree of Additional Reduction in CV Risk in Lowering LDL Below Minimum Target Levels [TNT]; NCT00327691) (J Am Coll Cardiol 2011;57:63–9) © 2011 by the American College of Cardiology Foundation

There has been much recent interest in the ability of nonlipid biomarkers associated with systemic inflammation, oxidative stress, tissue remodeling, and/or insulin resistance to predict adverse cardiovascular outcomes and to identify individuals at high risk of future coronary heart disease (CHD) events and stroke (1,2). The concentration of one of these, high-sensitivity C-reactive protein (hsCRP), predicts future cardiovascular events in apparently healthy individuals and in subjects treated with statins (3–5). The results of these studies have been used to support the argument that

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**Abbreviations and Acronyms**

**CHD** = coronary heart disease

**CVD** = cardiovascular disease

**HDL-C** = high-density lipoprotein cholesterol

**hsCRP** = high-sensitivity C-reactive protein

**LDL-C** = low-density lipoprotein cholesterol

**MCVE** = major cardiovascular event

**NT-proBNP** = N-terminal fragment of pro-B-type natriuretic peptide

the concentration of nonlipid biomarkers such as hsCRP should be included in algorithms designed to predict cardiovascular outcomes and to measure the efficacy of statin treatment (6). However, there are inconsistencies, with some studies finding that levels of nonlipid biomarkers have minimal predictive power beyond that of established CHD risk factors (7–9). We further address this issue by investigating how the concentrations of plasma lipids and nonlipid biomarkers relate to cardiovascular events in the TNT (Treating to New Targets) study.

## Methods

**Study design.** The study protocol and outcome measures for the TNT study have been published previously (10). In brief, patients with clinically manifest CHD commenced 8 weeks of open-label treatment with atorvastatin 10 mg/day. After this run-in period, 10,001 patients with low-density lipoprotein cholesterol (LDL-C) levels <3.4 mmol/l (<130 mg/dl) were randomized in a double-blind design to therapy with either 10 mg or 80 mg of atorvastatin/day. Patients were followed for a median of 4.9 years. The primary end point was the time to the first occurrence of a major cardiovascular event (MCVE), defined as CHD death; nonfatal, non-procedure-related myocardial infarction; resuscitated cardiac arrest; or fatal or nonfatal stroke. A full description of the nested case-control study population and biomarker analyses is presented in the Online Appendix (see also Table 1).

**Statistical methods.** Patient characteristics at randomization were compared between treatment groups of the main study and treatment groups of this substudy with a chi-square test for categorical variables and a Wilcoxon rank sum test for continuous variables. Similarly, characteristics of substudy patients at time of randomization were compared between those who did and those who did not experience a cardiovascular event during the study follow-up. Changes in biomarkers were tested with a signed rank test and compared between treatment groups with a Wilcoxon rank sum test. The association between on-treatment lipids and biomarker levels (at time of randomization and at 1 year) and primary end point was assessed with Cox proportional hazards after adjustment for age, sex, and treatment effect, with time to primary end point as the dependent variable for all patients and for patients within each treatment group. Treatment by biomarker interaction was examined separately in the same model with the addition of interaction term. Logistic regression was also used to confirm the findings observed with Cox proportional hazards. Independent variables included age, sex, treatment effect, and the $\log_2$ transformed biomarker level.

## Results

**Patient population.** Characteristics of patients in this substudy were similar to those in the total TNT population (Table 2). Characteristics of patients in the biomarker subgroup who experienced an MCVE and those who did not are also shown in Table 2 for the classical CHD risk factors and in Table 3 for the nonlipid biomarkers. The patients randomly selected as control subjects had similar characteristics compared with the rest of patients who did not have a clinical event ($p > 0.10$). At the time of randomization all participants had been taking atorvastatin at a dose of 10 mg/day for at least 8 weeks.

**Relationships of MCVEs to biomarker levels measured at time of randomization.** Table 4 shows the relationship between standard lipids and nonlipid biomarkers and risk of MCVE with Cox proportional hazards. In this analysis of the combined 10- and 80-mg atorvastatin groups, the concentrations of LDL-C, triglycerides, and high-density lipoprotein cholesterol (HDL-C) measured at randomization were all predictive of MCVEs. In contrast, almost none of the nonlipid biomarkers measured at the time of randomization predicted MCVEs. The only exception was osteopontin, with higher levels at randomization associated with a significantly lower risk of future MCVE. In addition to the analysis of the combined 10- and 80-mg atorvastatin groups, separate analyses were performed for each treatment group. These relationships between lipid levels at randomization and subsequent MCVEs in the separate treatment groups were consistent with those observed in the overall study population (Online Table 1). Similar results were obtained with logistic regression (Online Table 2) and upon exclusion of patients who had experienced fatal or nonfatal stroke during follow-up (Online Table 3).

**Effect of treatment on biomarker levels.** Significant changes from baseline in the levels of all lipids and most

**Table 1 Biomarkers Studied in TNT**

| Pathophysiological Role | Biomarkers Analyzed |
|---|---|
| Systemic inflammation | CRP |
| Macrophage recruitment/activity | MCP-1; neopterin; sICAM-1; sVCAM-1 |
| Oxidative stress | MPO; Lp-PLA2 |
| Tissue remodeling | MMP-9; osteopontin |
| Platelet/thrombosis | sCD40L; Lp(a) |
| Insulin resistance | Insulin; adiponectin; HMW adiponectin; HMW/total adiponectin (ratio); RAGE |
| Congestive heart failure | NT-proBNP |
| Kidney function | Cystatin C |

CRP = C-reactive protein; HMW = high molecular weight; Lp(a) = lipoprotein (a); Lp-PLA2 = lipoprotein-associated phospholipase A2; MCP = monocyte chemoattractant protein; MMP = matrix metalloproteinase; MPO = myeloperoxidase; NT-proBNP = N-terminal fragment of pro–B-type natriuretic peptide; RAGE = receptor for advanced glycation end products; sCD40L = soluble CD40 ligand; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular cell adhesion molecule; TNT = Treating to New Targets study.

**Table 2** Patients Characteristics at Time of Randomization

| Characteristic | Main Study by Treatment | | Substudy by Treatment | | Substudy by Event | | p Value* |
|---|---|---|---|---|---|---|---|
| | Atorvastatin 10 mg (n = 5,006) | Atorvastatin 80 mg (n = 4,995) | Atorvastatin 10 mg (n = 810) | Atorvastatin 80 mg (n = 717) | With Event (n = 507) | Without Event (n = 1,020) | |
| Age (yrs) | 60.9 (8.8) | 61.2 (8.8) | 60.8 (8.8) | 61.5 (8.5) | 62.6 (8.4) | 60.4 (8.7) | <0.0001 |
| Male (%) | 80.8 | 81.2 | 82.0 | 84.0 | 82.8 | 82.9 | 0.9606 |
| Risk factor (%) | | | | | | | |
| Current smoker | 13.4 | 13.4 | 14.0 | 14.1 | 18.5 | 11.8 | 0.0015 |
| Hypertension | 54.4 | 53.9 | 55.3 | 54.4 | 63.5 | 50.6 | <0.0001 |
| Diabetes | 15.0 | 15.0 | 15.9 | 15.6 | 23.3 | 12.1 | <0.0001 |
| Lipids (mg/dl) | | | | | | | |
| LDL-C | 98 (18) | 98 (17) | 97 (17) | 97 (17) | 100 (17) | 96 (17) | <0.0001 |
| Total cholesterol | 175 (24) | 175 (24) | 174 (24) | 174 (23) | 176 (25) | 173 (23) | 0.0296 |
| Triglycerides | 151 (72) | 151 (70) | 153 (72) | 148 (71) | 158 (78) | 147 (69) | 0.0145 |
| HDL-C | 47 (11) | 47 (11) | 46 (10) | 47 (11) | 45 (10) | 48 (11) | <0.0001 |

At the time of randomization, all participants had been taking 10 mg atorvastatin for at least 8 weeks. *The p value for patients that experienced an event versus those that did not in the biomarker subgroup. HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

nonlipid biomarkers were observed after 1 year in both the 10- and 80-mg atorvastatin groups (Fig. 1). There were no significant differences between the changes observed in the 2 treatment groups.

**Relationships between MCVEs and biomarker levels measured after 1 year of treatment.** After 1 year of treatment with atorvastatin, levels of LDL-C, HDL-C, and triglycerides remained significantly predictive of subsequent MCVEs in the combined groups and in the 10-mg atorvastatin group (Table 5). In the 80-mg atorvastatin group, it was only the HDL-C measured at 1 year that remained as a significant predictor of MCVEs. Almost none of the nonlipid biomarkers measured after 1 year of treatment were predictive of MCVEs in either treatment group, in contrast to the lipid levels. Changes in nonlipid biomarker levels between randomization and 1 year did not predict MCVEs in the total group or in either treatment group (data not shown). Similar results were obtained with logistic regression (Online Table 4) and upon exclusion of patients who had experienced fatal or nonfatal stroke during follow-up (Online Table 5).

## Discussion

This nested case-control substudy of the TNT trial was designed to investigate the ability of a number of lipid and nonlipid biomarkers to predict MVCEs in stable, statin-treated CHD patients. Although the ability of traditional

**Table 3** Non-Lipid Biomarker Levels at Time of Randomization

| Nonlipid Biomarker | Substudy by Treatment | | Substudy by Event | | p Value* |
|---|---|---|---|---|---|
| | Atorvastatin 10 mg (n = 810) | Atorvastatin 80 mg (n = 717) | With Event (n = 507) | Without Event (n = 1,020) | |
| Adiponectin (ng/ml) | 6,562 (4,821–9,411) | 6,593 (4,664–9,240) | 6,212 (4,651–9,338) | 6,702 (4,838–9,287) | 0.16 |
| HMW adiponectin (ng/ml) | 1,960 (1,180–3,155) | 1,929 (1,204–3,095) | 1,867 (1,188–2,948) | 1,969 (1,184–3,216) | 0.26 |
| HMW/total adiponectin | 0.292 (0.220–0.362) | 0.293 (0.229–0.368) | 0.293 (0.224–0.362) | 0.292 (0.225–0.367) | 0.81 |
| CRP (mg/l) | 1.7 (0.8–4.1) | 1.8 (0.8–3.8) | 1.8 (0.9–4.0) | 1.7 (0.7–3.8) | 0.08 |
| Cystatin C (ng/ml) | 779.7 (675.2–924.0) | 782.8 (677.8–915.5) | 789.4 (675.0–934.1) | 776.6 (676.9–912.6) | 0.51 |
| Insulin (μU/ml) | 12.0 (9.0–17.0) | 12.0 (9.0–17.5) | 12.0 (9.0–17.0) | 12.0 (9.0–18.0) | 0.41 |
| Lp-PLA2 (ng/ml) | 325.5 (259.5–387.5) | 323.0 (265.0–391.0) | 326.0 (264.0–389.0) | 324.0 (262.0–389.0) | 0.67 |
| Lp(a) (mg/ml) | 16 (5–40) | 13 (4–40) | 13 (4–39) | 15 (5–40) | 0.36 |
| MCP-1 (pg/ml) | 100 (75–130) | 99 (74–133) | 100 (77–131) | 100 (75–131) | 0.80 |
| MMP-9 (pg/ml) | 44,301 (30,679–65,702) | 43,619 (29,299–68,805) | 43,347 (30,370–70,114) | 44,524 (29,861–65,573) | 0.69 |
| MPO (pg/ml) | 22,237 (10,719–54,235) | 20,993 (10,324–57,628) | 22,077 (11,266–57,689) | 21,246 (10,116–56,275) | 0.31 |
| Neopterin (ng/ml) | 2.9 (2.3–3.6) | 2.9 (2.4–3.6) | 2.8 (2.3–3.6) | 2.9 (2.4–3.6) | 0.48 |
| NT-proBNP (fmol/ml) | 514 (407–661) | 503 (401–645) | 519 (405–645) | 505 (402–652) | 0.39 |
| Osteopontin (ng/ml) | 47.0 (32.2–60.5) | 46.4 (33.0–58.7) | 45.2 (29.9–59.2) | 47.5 (33.6–59.8) | 0.04 |
| RAGE (pg/ml) | 1,330 (1,025–1,831) | 1,318 (1,004–1,761) | 1,288 (1,011–1,861) | 1,341 (1,018–1,771) | 0.84 |
| sCD40L (pg/ml) | 4,033 (1,831–9,472) | 3,953 (1,978–9,566) | 3,918 (1,844–8,977) | 4,024 (1,879–9,987) | 0.37 |
| sICAM-1 (ng/ml) | 142 (108–184) | 146 (107–186) | 139 (103–185) | 145 (110–185) | 0.35 |
| sVCAM-1 (ng/ml) | 1,052 (0.885–1,275) | 1,048 (0.858–1,259) | 1,030 (0.855–1,271) | 1,056 (0.885–1,263) | 0.48 |

At the time of randomization, all participants had been taking 10 mg atorvastatin for at least 8 weeks. *The p value for patients that experienced an event versus those that did not in the biomarker subgroup. Abbreviations as in Table 1.

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**Table 4** Relationships of MCVEs to Biomarker Levels Measured at Time of Randomization

| | All Patients | | | Atorvastatin 10 mg | | | Atorvastatin 80 mg | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR* | 95% CI | p Value | HR* | 95% CI | p Value | HR* | 95% CI | p Value |
| Lipid biomarker | | | | | | | | | |
| LDL-C | 2.09 | 1.48–2.95 | <0.0001 | 2.27 | 1.42–3.64 | 0.0006 | 1.90 | 1.14–3.15 | 0.0134 |
| HDL-C | 0.35 | 0.26–0.47 | <0.0001 | 0.36 | 0.23–0.55 | <0.0001 | 0.33 | 0.23–0.51 | <0.0001 |
| Triglycerides | 1.27 | 1.10–1.46 | 0.0012 | 1.31 | 1.08–1.59 | 0.0068 | 1.21 | 0.99–1.50 | 0.0691 |
| Nonlipid biomarker | | | | | | | | | |
| Adiponectin | 0.96 | 0.87–1.05 | 0.3280 | 1.04 | 0.89–1.22 | 0.5940 | 0.91 | 0.82–1.01 | 0.0690 |
| HMW adiponectin | 0.98 | 0.91–1.07 | 0.6960 | 1.02 | 0.91–1.14 | 0.7450 | 0.94 | 0.84–1.06 | 0.3400 |
| HMW/total adiponectin | 1.01 | 0.91–1.13 | 0.8250 | 1.00 | 0.86–1.17 | 0.9950 | 1.03 | 0.88–1.19 | 0.7400 |
| CRP | 1.04 | 0.99–1.09 | 0.1090 | 1.07 | 1.01–1.14 | 0.0243 | 0.99 | 0.92–1.07 | 0.8170 |
| Cystatin C | 1.03 | 0.92–1.16 | 0.6200 | 1.06 | 0.91–1.24 | 0.4610 | 0.99 | 0.83–1.17 | 0.8700 |
| Insulin | 1.06 | 0.95–1.17 | 0.3200 | 1.12 | 0.97–1.29 | 0.1110 | 0.96 | 0.82–1.14 | 0.6540 |
| Lp-PLA2 | 0.99 | 0.80–1.23 | 0.9450† | 1.21 | 0.91–1.61 | 0.2000 | 0.77 | 0.56–1.06 | 0.1070 |
| Lp(a) | 0.99 | 0.94–1.05 | 0.8070 | 0.97 | 0.90–1.04 | 0.3830 | 1.02 | 0.95–1.11 | 0.5700 |
| MCP-1 | 1.02 | 0.91–1.14 | 0.7950† | 0.90 | 0.77–1.06 | 0.2110 | 1.15 | 0.98–1.35 | 0.0900 |
| MMP-9 | 0.99 | 0.91–1.08 | 0.8110 | 0.97 | 0.85–1.12 | 0.6790 | 1.00 | 0.89–1.14 | 0.9540 |
| MPO | 1.01 | 0.96–1.06 | 0.7930 | 0.99 | 0.93–1.06 | 0.8540 | 1.02 | 0.95–1.09 | 0.5920 |
| Neopterin | 1.00 | 0.85–1.17 | 0.9590 | 1.10 | 0.89–1.36 | 0.3890 | 0.89 | 0.71–1.12 | 0.3200 |
| NT-proBNP | 1.10 | 0.95–1.28 | 0.1850 | 1.07 | 0.87–1.31 | 0.5360 | 1.15 | 0.93–1.42 | 0.2060 |
| Osteopontin | 0.90 | 0.84–0.97 | 0.0030 | 0.88 | 0.80–0.97 | 0.0101 | 0.92 | 0.83–1.03 | 0.1420 |
| RAGE | 1.06 | 0.92–1.22 | 0.4270† | 1.22 | 1.01–1.46 | 0.0362 | 0.88 | 0.72–1.09 | 0.2450 |
| sCD40L | 0.98 | 0.93–1.02 | 0.3150 | 1.00 | 0.93–1.06 | 0.9040 | 0.95 | 0.89–1.02 | 0.1760 |
| sICAM-1 | 0.99 | 0.87–1.12 | 0.8360 | 1.03 | 0.86–1.23 | 0.7640 | 0.95 | 0.79–1.13 | 0.5460 |
| sVCAM-1 | 1.03 | 0.86–1.22 | 0.7570† | 1.31 | 0.99–1.72 | 0.0590 | 0.88 | 0.73–1.07 | 0.1930 |

At the time of randomization, all participants had been taking 10 mg atorvastatin for at least 8 weeks. *Hazard ratio (HR) associated with doubling the concentration and adjusting for age, sex, and treatment effect; †$p < 0.05$ ($p > 0.02$) for treatment by biomarker interaction.

CI = confidence interval; MCVE = major cardiovascular event; other abbreviations as in Tables 1 and 3.

lipid biomarkers to predict MCVEs was completely consistent with observations from a large number of previous studies, almost none of the emerging nonlipid biomarkers predicted the risk of MCVEs in the current analysis, a finding that seems to contrast with studies conducted in individuals not taking statins in which several of these nonlipid biomarkers have been reported to be independent predictors of incident MCVEs (11–13). This suggests that, in statin-treated patients with stable CHD, in contrast to standard lipids, nonlipid biomarkers have virtually no predictive value and should not be used to monitor the efficacy of statin treatment.

Several studies have shown that statins reduce plasma levels of markers associated with systemic inflammation, oxidative stress, tissue remodeling, and/or insulin resistance (4,5,14). In the present study—which is one of the most comprehensive studies on the topic, in terms of sample size and number of biomarkers studied—we found that most of the biomarkers plasma levels improved between the 8-week run-in period and 1 year. However, our results show that increasing atorvastatin dose to 80 mg did not result in additional changes in biomarkers levels. This finding seems to contrast with other trials performed in patients with CHD, such as the CAP (Comparative Atorvastatin Pleiotropic) effect study and the PROVE IT–TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction 22) study, in which a dose-response effect of statin on CRP levels was observed (80 mg atorvastatin vs. 40 mg pravastatin in the PROVE IT–TIMI 22 trial, and 80 mg atorvastatin vs. 10 mg atorvastatin in the CAP trial) (4,15). Additionally, in the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) trial, 80 mg atorvastatin provided a 36.4% decrease in CRP levels compared with 5.2% for patients treated with 40 mg pravastatin (16). Our results suggest that the biomarker that is most influenced by atorvastatin treatment is myeloperoxidase, which plasma levels increased by 20.7% and 25.6% for the 10- and 80-mg atorvastatin groups, respectively. Such an increase in myeloperoxidase levels upon statin therapy has already been observed by Meuwese et al. (17) in a sample of patients with heterozygous familial hypercholesterolemia treated with either atorvastatin 80 mg or simvastatin 40 mg. As for the predictive value of CRP, our results are not in line with those of the PROVE IT–TIMI 22 and A to Z (Aggrastat-to-Zocor) trials, in which plasma levels of CRP did predict cardiovascular disease (CVD) outcomes in statin-treated patients with acute coronary syndrome (4,18). However, it should be noted that, although these study populations were similar in terms of age, sex, diabetes, and hypertension prevalence as well as lipid levels, the proportion of smokers in the TNT trial was almost twice as low compared with the PROVE IT–TIMI 22 and A to Z study populations. This difference might explain, at least to a certain extent, the difference

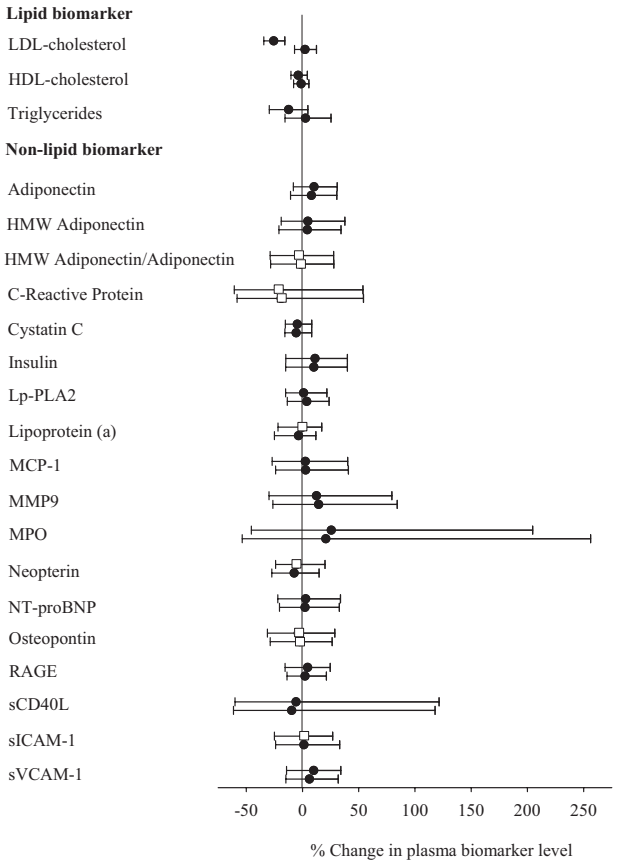


**Figure 1** **Percentage Change in Lipid and Nonlipid Biomarkers Levels After 1 Year of Treatment With Atorvastatin 10 or 80 mg**

Shown are median (interquartile range). Changes in biomarker levels did not differ between 10- and 80-mg atorvastatin groups (2-sided Wilcoxon rank sum test). For each biomarker, the **top bar** represents changes with 80 mg atorvastatin, and the **bottom bar** represents changes with 10 mg atorvastatin. **Black circles** represent significant changes from baseline to 1 year ($p < 0.05$ from signed rank test), and **white squares** represent nonsignificant changes. HDL-C = high-density lipoprotein cholesterol; HMW = high molecular weight; LDL-C = low-density lipoprotein cholesterol; Lp-PLA2 = lipoprotein-associated phospholipase A2; MCP = monocyte chemoattractant protein; MPO = myeloperoxidase; NT-proBNP = N-terminal fragment of pro–B-type natriuretic peptide; RAGE = receptor for advanced glycation end products; sCD40L = soluble CD40 ligand; sICAM = soluble intercellular adhesion molecule; sVCAM = soluble vascular cell adhesion molecule.

between CRP levels and cardiovascular outcomes observed across these studies.

Elevated plasma levels of inflammatory and oxidative stress markers are common in individuals with excess visceral adiposity and insulin resistance (19,20). Visceral adipocytes as well as the macrophages that infiltrate adipose tissue are major contributors to the plasma levels of many of the biomarkers we measured, including hsCRP (21,22). Because most of the study patients were older, were overweight or obese, or already had documented CHD, it is highly likely that most had an excess of visceral adipose tissue. Although statins have several documented pleiotropic effects, they have no effects on either body weight or body fat distribution. Thus, even though statins improved plasma levels of the studied biomarkers, there is no evidence that they have an impact on the likely biological sources of these biomarkers in the patients included in the TNT study. This might have had an influence on the observed relationships between biomarker levels and the risk of recurrent CHD. We believe that this analysis of TNT participants might have yielded different results if the intervention had been lifestyle modification instead of statin therapy.

The results of the present study do not question the relevance of systemic inflammation or inflammatory markers in the pathophysiology of CVD, for several reasons. We know from epidemiological studies that: 1) several markers of inflammation are independent CVD risk factors; and 2) initiating lipid-lowering therapy in individuals with elevated biomarker levels (at least hsCRP) brings clinical benefits (4,5). However, our observations suggest that such cardiovascular benefits are primarily attributable to the effects of statins on lipids rather than nonlipid biomarker levels. Future trials recording CHD outcomes in patients taking anti-inflammatory drugs (other than statins) are necessary to determine whether targeting inflammation is useful for CHD prevention (23).

In this case-control study, levels of traditional lipid biomarkers measured at randomization, when all subjects had already been taking 10 mg atorvastatin for at least 8 weeks, were predictive of future MCVEs in the combined group as well as in the 10- and 80-mg atorvastatin groups studied separately. Because the ability of LDL-C and HDL-C levels at randomization to predict future cardiovascular events was similar to what has been reported in many other studies, considerable confidence is provided in the validity of the approach used in this case-control study. However, it should be considered that these analyses were confined to patients participating to the TNT trial in whom additional informed consent was obtained. For instance, 51.5% of the cases recorded in the TNT cohort were included in the present analyses, which could have limited our power to detect associations between biomarkers and the risk of MCVE. In the absence of a complete dataset, we have used a nested case-control design. Although it could be argued that this might have limited the power to detect the true predictive value of the biomarkers tested, this type of design is increasingly being used to study causal relationships in cohort studies and has been demonstrated to be an efficient sampling method in well-defined cohorts (24). Case-control studies are often not based on well-defined patient populations, making it difficult on occasion to ensure that cases and control subjects are a representative sample of the population under study. In the current analysis, all cases were drawn from the well-defined TNT patient population, with the control subjects being randomly sampled from this same patient population. Also in support of the findings presented here, the predicted value of the lipid biomarkers for MCVEs (measured at randomization and after 1 year of

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**Table 5** Relationships of MCVEs to Biomarker Levels Measured After 1 Year of Treatment

| | All Patients | | | Atorvastatin 10 mg | | | Atorvastatin 80 mg | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR* | 95% CI | p Value | HR* | 95% CI | p Value | HR* | 95% CI | p Value |
| Lipid biomarker | | | | | | | | | |
| LDL-C | 1.43 | 1.09–1.87 | 0.0090 | 1.70 | 1.08–2.66 | 0.0209 | 1.31 | 0.93–1.84 | 0.1240 |
| HDL-C | 0.37 | 0.27–0.49 | <0.0001 | 0.36 | 0.23–0.56 | <0.0001 | 0.37 | 0.24–0.56 | <0.0001 |
| Triglycerides | 1.28 | 1.12–1.47 | 0.0003 | 1.36 | 1.13–1.63 | 0.0010 | 1.19 | 0.97–1.46 | 0.0990 |
| Nonlipid biomarker | | | | | | | | | |
| Adiponectin | 0.93 | 0.81–1.08 | 0.3630 | 1.00 | 0.82–1.23 | 0.9700 | 0.88 | 0.71–1.08 | 0.2130 |
| HMW adiponectin | 0.97 | 0.88–1.06 | 0.4660 | 1.04 | 0.89–1.18 | 0.7290 | 0.92 | 0.80–1.05 | 0.1940 |
| HMW/total adiponectin | 1.00 | 0.87–1.15 | 0.9770 | 1.08 | 0.87–1.33 | 0.5010 | 0.94 | 0.78–1.13 | 0.5170 |
| CRP | 1.02 | 0.96–1.08 | 0.5240 | 1.06 | 0.98–1.15 | 0.1640 | 0.97 | 0.89–1.06 | 0.5460 |
| Cystatin C | 0.95 | 0.84–1.06 | 0.3590† | 1.08 | 0.89–1.30 | 0.4560 | 0.84 | 0.72–0.97 | 0.0190 |
| Insulin | 0.99 | 0.87–1.13 | 0.8890 | 0.92 | 0.76–1.12 | 0.4010 | 1.05 | 0.88–1.27 | 0.5850 |
| Lp-PLA2 | 1.06 | 0.82–1.37 | 0.6610 | 1.27 | 0.88–1.84 | 0.2110 | 0.89 | 0.62–1.29 | 0.5420 |
| Lp(a) | 0.97 | 0.91–1.03 | 0.3430 | 0.93 | 0.85–1.01 | 0.1000 | 1.02 | 0.93–1.11 | 0.7150 |
| MCP-1 | 1.08 | 0.94–1.24 | 0.2860 | 1.01 | 0.82–1.24 | 0.9510 | 1.15 | 0.95–1.39 | 0.1580 |
| MMP-9 | 1.00 | 0.90–1.11 | 0.9780 | 1.00 | 0.85–1.17 | 0.9940 | 1.00 | 0.85–1.16 | 0.9440 |
| MPO | 0.99 | 0.93–1.06 | 0.7860 | 1.02 | 0.94–1.12 | 0.6140 | 0.96 | 0.87–1.05 | 0.3640 |
| Neopterin | 1.12 | 0.93–1.35 | 0.2430 | 1.19 | 0.92–1.55 | 0.1870 | 1.04 | 0.80–1.37 | 0.7590 |
| NT-proBNP | 1.02 | 0.85–1.22 | 0.8390 | 1.13 | 0.86–1.47 | 0.3830 | 0.93 | 0.73–1.19 | 0.5790 |
| Osteopontin | 1.01 | 0.91–1.11 | 0.9080 | 1.04 | 0.90–1.20 | 0.6090 | 0.98 | 0.86–1.11 | 0.7590 |
| RAGE | 1.02 | 0.87–1.21 | 0.7850 | 1.08 | 0.85–1.36 | 0.5270 | 0.97 | 0.76–1.23 | 0.7780 |
| sCD40L | 1.03 | 0.97–1.09 | 0.3850 | 1.04 | 0.96–1.12 | 0.3540 | 1.01 | 0.93–1.10 | 0.7480 |
| sICAM-1 | 1.00 | 0.85–1.19 | 0.9870 | 1.00 | 0.78–1.28 | 1.0000 | 1.00 | 0.79–1.27 | 0.9800 |
| sVCAM-1 | 0.95 | 0.76–1.19 | 0.6700 | 1.01 | 0.73–1.39 | 0.9780 | 0.91 | 0.67–1.22 | 0.5070 |

*The HR associated with doubling the concentration and adjusting for age, sex, and treatment effect; †p = 0.05 for treatment × biomarker interaction. Individuals who experienced an MCVE within 1 year of follow-up (n = 157) were not included in the present analyses.

Abbreviations as in Tables 1, 2, 3, and 4.

treatment) were similar in the samples selected in this nested case-control study and the entire TNT sample set. Thus, results from this biomarker study are highly likely to be applicable to patients similar to those studied in the TNT trial. The TNT trial did not include a placebo group, because all participants received active treatments. It was thus not possible to make comparisons with untreated patients. As a consequence, it must be emphasized that our data should not be interpreted as a negation or a contradiction of the results of studies showing the ability of biomarkers to predict MCVEs in other populations or in asymptomatic individuals. Finally, it should be taken into consideration that the assays that we have used for the measurement of CRP and N-terminal fragment of pro–B-type natriuretic peptide (NT-proBNP) have not been clinically validated. However, a cross-validation study demonstrated that the human CVD Panel 2 LINCOplex kit from Millipore (St. Charles, Missouri) (used in the present study) was highly correlated with the clinically validated Dade Behring (Deerfield, Illinois) assay (correlation coefficients of r = 0.91 for baseline samples [n = 150] and r = 0.93 for year-1 samples [n = 116]). As for NT-proBNP, it must be acknowledged that the assay used in this study (Biomedica, Vienna, Austria) is based on a different principle than the clinically validated assay from Roche Diagnostics (Basel, Switzerland), because the assays recognize different epitopes/fragments of NT-proBNP. However, even if both assays have been shown to have a comparable ability to detect the presence of heart failure (25), our results cannot completely rule out the potential presence of a positive association between NT-proBNP and cardiovascular events in statin-treated patients.

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**Key Words:** biomarkers ■ coronary heart disease ■ inflammation ■ lipids ■ oxidative stress ■ statins.

## APPENDIX

**For supplementary data and tables, please see the online version of this article.**