Association of Nitrotyrosine Levels With Cardiovascular Disease and Modulation by Statin Therapy

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NITRIC OXIDE IS A VASODILATOR AND INHIBITOR OF PLATELET AGGREGATION, LEUKOCYTE ADHESION, AND SMOOTH MUSCLE CELL PROLIFERATION.1-3 However, under pathological conditions, nitric oxide may be converted into potent nitrating oxidants that promote oxidative damage, cell injury, and conversion of low-density lipoprotein (LDL) into an atherogenic form.4-5 One pathway for generating nitric oxide–derived oxidants involves interaction with superoxide anion, leading to formation of peroxynitrite. Peroxynitrite is a potent oxidant that promotes nitration of protein tyrosine residues producing a distinctive “molecular fingerprint” for nitric oxide–derived oxidants, nitrotyrosine.6,7 An alternative mechanism for generating nitric oxide–derived oxidants involves myeloperoxidase,7,11 a leukocyte-derived enzyme enriched in atherosclerotic lesions that serves as an

Context Formation of nitric oxide–derived oxidants may serve as a mechanism linking inflammation to development of atherosclerosis. Nitrotyrosine, a specific marker for protein modification by nitric oxide–derived oxidants, is enriched in human atherosclerotic lesions and low-density lipoprotein (LDL) recovered from human atheroma.

Objectives To determine whether systemic levels of nitrotyrosine are associated with the prevalence of coronary artery disease (CAD) and are modulated by hydroxymethylglutaryl coenzyme-A reductase inhibitor (statin) therapy.

Design, Setting, and Patients A case-control and interventional study at 2 urban tertiary-care referral centers; recruitment for each was from June 1, 2001, until January 1, 2002. For the case-control study, 100 case-patients with established CAD and 108 patients with no clinically evident CAD were recruited consecutively. In the interventional study, participants aged 21 years or older with hypercholesterolemia (LDL cholesterol ≥130 mg/dL [≥3.5 mmol/L]) underwent nutrition and exercise counseling. Those whose levels did not decrease with 6 to 8 weeks were enrolled in the study (n=35). For 12 weeks, they received 10 mg/d of oral atorvastatin therapy.

Main Outcome Measures In the case-control study, the association between systemic levels of protein-bound nitrotyrosine, CAD risk, and presence of CAD. In the interventional study, the change in nitrotyrosine, lipoprotein, and C-reactive protein (CRP) levels.

Results Nitrotyrosine levels were significantly higher among patients with CAD (median 9.1 µmol/mol [interquartile range, 4.8-13.8 µmol/mol] tyrosine vs 5.2 µmol/mol [interquartile range, 2.2-8.4 µmol/mol]; P<.001). Patients in the upper quartile of nitrotyrosine (29%; P<.001) had a higher odds of CAD compared with those in the lowest quartile (unadjusted odds ratio, 6.1; 95% confidence interval, 2.6-14.0; P<.001). In multivariate models adjusting for Framingham Global Risk Score and CRP, upper quartiles of nitrotyrosine remained associated with CAD (odds ratio, 4.4; 95% confidence interval, 1.8-10.6; P<.001). Statin therapy reduced nitrotyrosine levels significantly (25%; P<.001) with a magnitude similar to reductions in total cholesterol levels (25%; P<.001) and LDL particle number (29%; P<.001) yet were independent of alterations in lipoproteins and inflammatory markers like CRP.

Conclusions The findings from this preliminary study indicate that nitrotyrosine levels are associated with the presence of CAD and appear to be modulated by statin therapy. These results suggest a potential role for nitric oxide–derived oxidants as inflammatory mediators in CAD and may have implications for atherosclerosis risk assessment and monitoring of anti-inflammatory actions of statins.

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Financial Disclosure: Dr Hazen is named as co-inventor on pending patents filed by the Cleveland Clinic Foundation that relate to the use of biomarkers for inflammatory and cardiovascular diseases.

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NAD(P)H oxidase complexes. Statins anion formation within vascular tissue is mediated by enzymatic sources for superoxide formation. The net effect of statin therapy and enhanced nitric oxide production is the recruitment of G-protein Rho leads to increased levels of nitric oxide–derived oxidants and nitrotyrosine. Some of the patients with CAD also had peripheral arterial disease (PAD), defined as an ankle-brachial index less than 0.9, intermittent claudication, and/or documented stenoses of peripheral or carotid arteries by angiography, magnetic resonance imaging, or ultrasound. Controls had no clinical history or symptoms suggestive of CAD or PAD. All participants gave written informed consent, and the institutional review board of Boston Medical Center approved the study protocol.

Prospective Intervention Study
We enrolled 35 consecutive patients from the Preventive Cardiology Clinic at the Cleveland Clinic Foundation from June 2001 until January 2002. Patients who were at least 21 years old, had no clinical evidence of CAD, PAD, or diabetes mellitus, were naive to statin therapy, and had low-density lipoprotein cholesterol (LDL-C) levels that were 130 mg/dL or higher (≥3.3 mmol/L) received counseling on nutritional and exercise interventions. If after 6 to 8 weeks LDL-C remained at 130 mg/dL or higher (≥3.3 mmol/L), patients were eligible for enrollment in the study. Fasting morning plasma samples were collected prior to initiation of therapy (baseline) and following 12 weeks of atorvastatin therapy (10 mg/d). All patients enrolled completed the study. Exclusion criteria included liver disease, renal insufficiency, or changes in medical therapy during the treatment period. All patients gave written informed consent, and the institutional review board at the Cleveland Clinic Foundation approved the study protocol.

Laboratory Analysis
General. Blood samples were collected into serum separator tubes (case-control study) or EDTA tubes (interventional study) from patients who had fasted overnight. Samples were centrifuged at 3500 rpm for 10 minutes, plasma/serum were recovered, and aliquots were stored at −80°C until analysis. Personnel blinded to clinical data performed all laboratory measurements. Lipoprotein/lipid profiles and high-sensitivity C-reactive protein (CRP) measurements were performed as previously described. Nitrotyrosine. Protein-bound nitrotyrosine levels were determined by stable isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry-based methods using an ion trap mass spectrometer (LCQ Deca, ThermoFinigann, San Jose, Calif), as previously described. Synthetic 3-nitro-[13C9,15N1]tyrosine (2 pmol) and [13C9,15N1]tyrosine (2 mmol) were added to protein pellets both as internal standards and to simultaneously monitor nitrotyrosine, tyrosine, and potential artificial formation of nitrotyrosine during analyses.

Statistical Analysis
Case-Control Study. Nitrotyrosine levels were not normally distributed (Shapiro-Wilk test). Consequently, quartile-based methods were used for analyses, and summary measures were presented as median and interquartile range. Comparisons between cases and controls were made with χ² tests for categorical measures and Wilcoxon rank-sum tests for continuous measures. Trends were assessed with Cochran-Armitage tests.

Logistic regression models (SAS System, SAS Institute, Cary, NC) were used to determine whether CAD is associated with increased systemic levels of nitrotyrosine and whether statin therapy would reduce these levels.

METHODS
Case-Control Study
We enrolled 208 individuals from 2 venues in Boston, Mass, from June 2001 until January 2002. Consecutive patients presenting to the cardiology section of the Boston Medical Center were enrolled. Simultaneously (and to increase recruitment of controls), consecutive area residents responding to advertisements in a community newspaper were recruited. The 100 case patients had a history of CAD, defined as documented myocardial infarction, coronary artery bypass graft surgery, percutaneous coronary intervention, or a stenosis of 50% or greater in 1 or more major coronary vessels on angiography. Some of the patients with CAD also had peripheral arterial disease (PAD), defined as an ankle-brachial index less than 0.9, intermittent claudication, and/or documented stenoses of peripheral or carotid arteries by angiography, magnetic resonance imaging, or ultrasound. Controls had no clinical history or symptoms suggestive of CAD or PAD. All participants gave written informed consent, and the institutional review board of Boston Medical Center approved the study protocol.

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to calculate odds ratios (ORs) associated with the second, third, and highest quartile of nitrotyrosine compared with the lowest quartile for the indicated outcomes. Single adjustments were made for individual traditional CAD risk factors (age, sex, diabetes, hypertension, smoking [ever or current]), family history, total cholesterol, LDL-C, high-density lipoprotein cholesterol [HDL-C], triglycerides, CRP), and a modified Framingham Global Risk Score,10 alone and with CRP. Hosmer-Lemeshow goodness-of-fit tests were used to evaluate appropriate model fit. Associations among continuous variables were assessed with use of Spearman rank-correlation coefficient. Associations among categorical variables were assessed using Wilcoxon rank-sum tests.

The study was planned to have at least 100 patients per group, based on a logistic regression power calculation demonstrating that 198 patients would provide 80% power ($\alpha = .05$) to detect an OR of 2.0 for elevated nitrotyrosine (upper quartile).

Interventional Study. Wilcoxon rank-sum test was used to analyze the differences between measurements at baseline and 12 weeks. Spearman rank-correlation coefficients were used to assess associations between both baseline and atorvastatin-induced changes in nitrotyrosine levels, lipoprotein profile measures, and CRP levels. Approximate 95% confidence intervals (CIs) were found using Fisher $r$-to-$z$ transform. Multiple regression analyses were performed to determine factors associated with changes in nitrotyrosine levels.

RESULTS

Case-Control Study

Patient Demographics. Baseline characteristics of study participants are shown in Table 1. As expected, patients with CAD were older, more likely to be men, and more likely to have hypertension, diabetes mellitus, or family history of CAD. Patients with CAD also had increased triglyceride levels and CRP levels, and they were more likely to use lipid-lowering drugs and other cardiovascular medications.

Nitrotyrosine Levels and CAD. Nitrotyrosine levels were higher in patients with CAD compared with controls (median values, 9.1 µmol/mol tyrosine vs 5.2 µmol/mol tyrosine, respectively; $P < .001$; Table 1). Rates of CAD increased with higher nitrotyrosine quartiles (26% vs 58%, lowest vs highest quartiles; $P < .001$ for trend). Patients in the highest quartile of nitrotyrosine levels had increased risk of CAD compared with patients in the lowest quartile (Table 2; the unadjusted nitrotyrosine fourth quartile OR, 6.1; 95% CI, 2.6-14.0; $P < .001$). CAD rates were also higher with increasing CRP quartiles (25% vs 50%, lowest vs highest quartiles; $P < .001$ for trend). The proportion of patients with CAD was higher among those in the upper quartile of both nitrotyrosine and CRP levels compared with patients in the lower quartiles (76% vs 7%; $P < .001$).

Nitrotyrosine Levels and CAD Risk Factors. Nitrotyrosine levels correlated with age ($r = 0.14$, $P = .03$), fasting triglycerides ($r = 0.14$, $P = .03$), and CRP levels ($r = 0.15$, $P = .02$); however, these associations were small in magnitude and accounted for less than 5% of the observed variance in nitrotyrosine. There was no significant correlation between nitrotyrosine and LDL-C, HDL-C, or total cholesterol. Participants with diabetes had higher nitrotyrosine levels than those who did not have diabetes (median values, 9.6 µmol/mol tyrosine vs 5.7 µmol/mol tyrosine, respectively; $P < .001$).

Adjusted Models for Nitrotyrosine and CAD. Nitrotyrosine levels remained significantly associated with CAD following individual adjustments for age; sex; history of diabetes; current smoking; history of hypertension; and levels of HDL-C, LDL-C, triglyceride, and CRP with minimal changes observed in adjusted ORs and CIs (data not shown). After adjustment for the Framingham Global Risk Score, nitrotyrosine remained a robust predictor of presence of CAD (Table 2, Model 1; adjusted nitro-

Table 1. Baseline Characteristics by Coronary Artery Disease Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control Patients (n = 108)</th>
<th>Patients With CAD (n = 100)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>44 (35-53)</td>
<td>59 (53-67)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Women, No. (%)</td>
<td>76 (70)</td>
<td>46 (46)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index, median (IQR)</td>
<td>28 (24-33)</td>
<td>27 (24-33)</td>
<td>.80</td>
</tr>
<tr>
<td>Risk factors, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (3)</td>
<td>34 (34)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36 (33)</td>
<td>63 (63)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>18 (17)</td>
<td>48 (48)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>53 (49)</td>
<td>73 (73)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cholesterol, median (IQR), mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>199 (180-225)</td>
<td>196 (168-212)</td>
<td>.03</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>59 (46-67)</td>
<td>53 (39-67)</td>
<td>.58</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>119 (99-147)</td>
<td>99 (49-128)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglycerides, median (IQR), mg/dL</td>
<td>106 (68-148)</td>
<td>148 (125-198)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/dL</td>
<td>0.19 (0.08-0.46)</td>
<td>0.49 (0.32-1.50)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nitrotyrosine, median (IQR), µmol/mol tyrosine</td>
<td>5.2 (2.2-8.4)</td>
<td>9.1 (4.8-13.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Therapy, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>5 (5)</td>
<td>84 (84)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>10 (9)</td>
<td>40 (40)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>2 (2)</td>
<td>3 (3)</td>
<td>.59</td>
</tr>
<tr>
<td>$\beta$-Blockers</td>
<td>12 (11)</td>
<td>72 (72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>6 (6)</td>
<td>18 (18)</td>
<td>.005</td>
</tr>
<tr>
<td>Nitrates</td>
<td>3 (3)</td>
<td>15 (15)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Statins</td>
<td>3 (3)</td>
<td>39 (39)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, coronary artery disease; IQR, interquartile range.

SI conversion factors: To convert high-density lipoprotein, low-density lipoprotein, and total cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglycerides, multiply by 0.0113.
tyrosine 4th quartile OR, 5.4; 95% CI, 2.0-14.3; P< .001). Addition of CRP to the model had little effect on the OR for nitrotyrosine as a predictor of CAD status (Table 2, Model 2; adjusted nitrotyrosine fourth quartile OR, 4.4; 95% CI, 1.8-10.6; P< .001). Likelihood ratio tests confirmed that introducing nitrotyrosine to multivariable prediction models that included established markers of cardiovascular risk (eg, Model 2, Table 2) significantly added to prediction for presence of CAD (χ² = 10.42; P< .001).

Nitrotyrosine Levels and Atherosclerosis Burden. We also examined whether nitrotyrosine levels correlate with clinical evidence of atherosclerotic burden (ie, determine if the correlation of nitrotyrosine levels with atherosclerosis is even stronger in patients with more extensive atherosclerosis [CAD plus PAD]). Patients with CAD plus PAD demonstrated increases in prevalence of atherosclerosis with increasing nitrotyrosine quartiles (3% vs 46%, lowest vs highest quartiles; P< .001 for trend). Within this atherosclerosis-laden group, nitrotyrosine served as the strongest independent predictor associated with atherosclerosis risk following multivariable adjustments with Framingham Global Risk Score and CRP (adjusted nitrotyrosine fourth quartile OR, 25.4; 95% CI, 2.8-274; P< .001 [Table 2]; adjusted Framingham Global Risk Score OR, 1.25; 95% CI, 1.15-1.36; P< .001; adjusted CRP fourth quartile OR, 5.0; 95% CI, 2.1-16.6; P< .001).

### Intervventional Study

In the case-control study of the entire cohort (n = 208), nitrotyrosine levels demonstrated a tendency toward being lower in patients taking statins (P= .06), suggesting that statin therapy may reduce nitrotyrosine levels. This was directly assessed in an interventional study comprised of 35 patients with a mean (SD) of 54 (10) years, 49% of whom were men. Table 3 shows the lipid and lipoprotein levels, CRP, and nitrotyrosine levels at baseline and after taking orally 10 mg/d of atorvastatin for 12 weeks. Atorvastatin treatment reduced total cholesterol levels by 25%, LDL-C by 39%, and apolipoprotein B-100 by 29%. Statin-induced reductions in plasma nitrotyrosine levels (25%; P= .02) were similar in magnitude to decreases in total cholesterol and LDL particle number (ie, apolipoprotein B-100). A nonsignificant trend toward statin-induced reductions in CRP levels was also observed (11% reduction; P=.10).

No significant correlations were noted between baseline levels of nitrotyrosine, lipid parameters, and CRP. Furthermore, no significant correlations were noted between statin-

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### Table 2. Odds Ratios of Cardiovascular Disease Risk According to Quartiles of Nitrotyrosine

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrotyrosine, µmol /mol tyrosine</td>
<td>&lt;3.6</td>
<td>3.6 – 6.31</td>
<td>6.32 – 10.04</td>
<td>≥10.05</td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2

- **CAD, Cases (n = 100), Controls (n = 108)**
- **No. of cases**: 17 19 26 38
- **No. of controls**: 38 29 27 14
- **Odds ratio, (95% CI)**
  - **Unadjusted**: 1.0 1.4 (0.6-3.3) 2.2 (1.0-4.7) 6.1 (2.6-14.0) < .001
  - **Model 1**: 1.0 2.3 (0.8-6.3) 2.1 (0.8-5.3) 5.4 (2.0-14.3) < .001
  - **Model 2**: 1.0 2.3 (0.3-2.0) 1.1 (0.4-3.0) 4.4 (1.8-10.6) < .001

#### Table 3. Lipid Levels, High-Sensitivity C-Reactive Protein, and Nitrotyrosine at Baseline and After 12 Weeks of Treatment With Atorvastatin*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (n = 35)</th>
<th>12 Weeks (n = 35)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrotyrosine (µmol/mol tyrosine)</td>
<td>15 (7)</td>
<td>11 (5)</td>
<td>.02</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
<td>0.26 (0.32)</td>
<td>0.23 (0.33)</td>
<td>.10</td>
</tr>
<tr>
<td>Cholesterol, mg/dL Total</td>
<td>253 (27)</td>
<td>190 (28)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>56 (12)</td>
<td>58 (12)</td>
<td>.21</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>169 (22)</td>
<td>103 (20)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>146 (60)</td>
<td>132 (81)</td>
<td>.22</td>
</tr>
<tr>
<td>Apolipoprotein B-100, mg/dL</td>
<td>135 (17)</td>
<td>96 (21)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Conversion factors: To convert high-density lipoprotein, low-density lipoprotein, and total cholesterol from mg/dL to mmol/L, multiply by 0.0259; triglycerides, multiply by 0.0113.

*P values represent comparisons between mean baseline and 12-week levels for the indicated variables.
induced changes in nitrotyrosine vs changes in lipoprotein and inflammatory markers including total cholesterol level (95% CI; ρ = −0.23 to 0.43), LDL-C (95% CI; ρ = −0.2 to 0.45), HDL-C (95% CI; ρ = −0.18 to 0.47), or CRP (95% CI; ρ = −0.22 to 0.44). In multivariable regression analysis, there was no significant association between change in nitrotyrosine levels and changes in levels of total cholesterol, LDL-C, HDL-C, and CRP (F-ratio = 0.71; P = .60).

**COMMENT**

The results of these preliminary studies suggest that nitrotyrosine, a marker specific for protein modification by nitric oxide–derived oxidants, may serve as an inflammatory marker for CAD. Systemic levels of protein-bound nitrotyrosine were associated with presence of CAD even following multivariable adjustments for traditional CAD risk factors and CRP. Importantly, statin therapy promoted significant reductions in nitrotyrosine levels that were similar in magnitude to reductions in total cholesterol and LDL particle number. Moreover, reductions in nitrotyrosine promoted by statin therapy were independent of reductions in lipid parameters and CRP. Taken together, these results suggest that nitrotyrosine measurements may prove useful both in assessing CAD status and for monitoring the anti-inflammatory effects of statins.

Numerous lines of evidence support potential links between formation of nitric oxide–derived oxidants and development of CAD. Current evidence suggests that an imbalance between superoxide and nitric oxide formation within diseased artery walls leads to a functional deficiency of nitric oxide, and consequent generation of nitric oxide–derived oxidants such as peroxynitrite and myeloperoxidase–generated reactive nitrogen species. Enhanced formation of superoxide in diseased artery walls may occur through vascular, endothelial (eg, nox), and leukocyte–derived NAD(P)H oxidase complexes, as well as nitric oxide synthase that is “uncoupled.” Superoxide thus formed may interact with nitric oxide, resulting in formation of nitrating oxidants. Similarly, myeloperoxidase, a leukocyte–derived heme protein enriched in human atheroma, catalytically consumes nitric oxide as a physiological substrate. Organ chamber studies and studies with myeloperoxidase–dependent pathways for generating nitric oxide–derived oxidants, as monitored by nitrotyrosine formation. One consequence of these reactions appears to be the oxidative conversion of LDL into a high uptake form for macrophages, leading to cholesterol accumulation and foam cell formation. Alternative mechanisms have linked nitric oxide–derived oxidants to activation of matrix metalloproteases and development of unstable plaques and development of prothrombic states. The potential contributions of nitric oxide–derived oxidants to CAD development are thus numerous and varied.

In this study, therapy with low-dose atorvastatin significantly reduced nitrotyrosine levels. Statins promote systemic effects that extend beyond simply lowering cholesterol levels. Statin-induced inhibition in superoxide formation has been shown in cultured vascular smooth muscle cells. We therefore hypothesized that significant reductions in nitrotyrosine would be noted since pathways leading to formation of nitric oxide–derived oxidants invariably require superoxide generation. The mechanism for decreased superoxide formation appears to involve inhibition of isoprenylation of the protein Rac, a key NAD(P)H oxidase component that normally requires isoprenylation for appropriate translocation to the plasma membrane during cell stimulation. Thus, in contrast to the modest alterations in CRP typically noted relative to those observed for lipoprotein and cholesterol levels, these results demonstrated that nitrotyrosine reductions were comparable in magnitude to those noted for total cholesterol or LDL particle number with administration of low-dose statin (Table 3). The growing appreciation of the pleiotropic actions of statins has underscored the requirement for new measures that quantify the anti-inflammatory properties of this widely used class of drugs. Our study suggests that systemic nitrotyrosine levels may serve as an independent measure of the anti-inflammatory actions of statins.

A corollary to these findings is that low-dose atorvastatin therapy promotes potent systemic antioxidant effects by suppressing formation of nitric oxide–derived oxidants. Further studies of the systemic antioxidant actions promoted by statin therapy are warranted. Recent randomized trials with antioxidant vitamins, particularly α-tocopherol, have failed to demonstrate benefit against cardiovascular disease, and it is notable that α-tocopherol is relatively ineffective at blocking the effects of nitric oxide–derived oxidants. It is tempting to speculate that interventional studies with statins, which have repeatedly demonstrated clinical benefits, have in fact also been “antioxidant” trials that used therapeutic agents able to suppress generation of more clinically relevant nitric oxide–derived oxidants. Elevated nitrotyrosine levels in patients with diabetes were recently reported, a finding also observed in our cohort. Postprandial elevations in nitrotyrosine levels following consumption of a high fat or high glucose meal that were attenuated following simvastatin therapy were also recently reported. Although nitrotyrosine enrichment in human atherosclerotic lesions is well known from both immunohistochemical and mass spectrometry–based studies, our study is the first, to our knowledge, that di-
directly correlates systemic levels of nitrotyrosine with presence of CAD and response to statin therapy. The cross-sectional (rather than longitudinal) design of the case-control study and the pilot nature of the intervention study are important limitations of our study. However, the results point toward promising potential clinical utility in use of nitrotyrosine levels as an adjunct for CAD risk stratification and monitoring of anti-inflammatory actions of statin therapy. Further evaluation of nitrotyrosine levels as a predictor of future cardiovascular events and outcomes and as a means of monitoring risk reduction attendant with statin therapy are warranted.

**Author Contributions:** Study concept and design: Sprecher, Vita, Hazen. Acquisition of data: Shishebor, Aviles, Brennan, Fu, Gokce, Keaney, Vita, Hazen. Analysis and interpretation of data: Shishebor, Aviles, Brennan, Fu, Goormastic, Pearce, Gokce, Keaney, Penn, Sprecher, Vita, Hazen. Drafting of the manuscript: Shishebor, Aviles, Brennan, Pearce, Gokce, Penn, Vita, Hazen. Critical revision of the manuscript for important intellectual content: Shishebor, Aviles, Brennan, Fu, Goormastic, Keaney, Penn, Sprecher, Vita, Hazen. Obtained funding: Vita, Hazen. Administrative, technical, or material support: Brennan, Fu, Gokce, Hazen. Study supervision: Gokce, Keaney, Hazen.

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