Accumulation of Zinc in Human Atherosclerotic Lesions Correlates With Calcium Levels but Does Not Protect Against Protein Oxidation

Nadina Stadler, Naomi Stanley, Sylvia Heeneman, Vladimir Vacata, Mat J.A.P. Daemen, Paul G. Bannon, Johannes Waltenberger, Michael J. Davies

Objective—Oxidized lipids and proteins, and decreased antioxidant levels, have been detected in human atherosclerotic lesions, with oxidation catalyzed by iron and copper postulated to contribute to lesion development. Zinc has been postulated to displace iron from critical sites and thereby protect against damage. In this study, metal ion and protein oxidation levels were quantified in human carotid and abdominal artery specimens containing early to advanced lesions, to determine whether zinc concentrations correlate inversely with iron levels and protein oxidation.

Methods and Results—Metal ions were quantified by EPR and inductively coupled plasma mass spectroscopy. Native and oxidized protein side-chains were quantified by high-performance liquid chromatography. Elevated levels of zinc (≈6-fold) were detected in advanced lesions compared to healthy tissue or early lesions. Zinc did not correlate negatively with iron or copper levels suggesting that zinc does not displace these metal ions. Highly significant positive correlations (P<0.005) were detected between zinc and calcium levels.

Conclusions—Zinc did not correlate with low iron levels and reduced protein oxidation. These data indicate that zinc does not prevent protein oxidation in advanced lesions. The reported protective effect of zinc accumulation is proposed to be associated with lesion calcification. (Arterioscler Thromb Vasc Biol 2008;28:000-000.)

Key Words: atherosclerosis ■ iron ■ zinc ■ protein oxidation ■ calcium
aortic lesion cross-sectional area, and reduce a number of markers of cholesterol and lipid oxidation. These data are consistent with zinc having an antiatherogenic effect, with this postulated to occur via a reduction in iron-catalyzed radical reactions. High zinc concentrations have also been reported to reduce intimal hyperplasia in a rat carotid artery balloon-injury model.

Zinc may modulate biological effects via a range of alternative mechanisms than prevention of oxidation, such as via the stabilization of zinc-finger genes involved in lipid metabolism, via peroxisome proliferator activated receptor signaling, by modulating transcription factors such as NF-κB and AP-1, and by interference with caspase expression and apoptosis.

In the light of this conflicting data on the role of zinc in atherosclerosis and uncertainty about the mechanism of the reported effects, we investigated the levels of zinc, iron, calcium, and copper in carotid and abdominal atherosclerotic lesions (both postmortem and from endarterectomy operations) and examined whether metal ion levels correlate with the extent of protein oxidation.

**Methods**

**Postmortem Tissue Specimens**

Fifty-three human specimens (26 carotid artery, Car; 27 abdominal aorta, AbAo) were obtained from 16 donors aged 53 to 91, autopsied at the University Hospital Maastricht, via the Maastricht Pathology Tissue Collection. The collection, storage, and use of tissue and patient data were performed in accordance with the “Code for Proper Use of Secondary Human Tissue in the Netherlands.” In most cases, 2×1.5 cm consecutive ring segments of the common right Car (1.5 cm proximal of the carotid bifurcation) and 2×2 cm segments of AbAo were removed and rinsed. Four-mm slices from the end of each segment were cut on ice, fixed in 10% phosphate-buffered formalin (pH 7.4), embedded in paraffin, stained, and classified according to Virmani et al. Remaining tissue was stored at 80°C. Sections were classified as: nondiseased, early nonatherosclerotic intimal lesions (intimal thickening and intimal xanthoma; INT); intermediate progressive atherosclerotic lesions (pathological intimal thickening; PIT); and advanced lesions (ADV) characterized by thick or thin fibrous cap atheromas containing either a well-formed necrotic core, calcification, fibrous tissue, or lesions containing a thrombus, which included ruptured plaques, or those with intraplaque hemorrhage.

**Carotid Endarterectomy Specimens**

Forty-four human artery samples were obtained after informed consent and ethics committee approval (Royal Prince Alfred Hospital, Sydney, Australia) from symptomatic patients undergoing carotid endarterectomy operations. Twelve mammary and 4 radial arterial specimens (from bypass and transplantation operations) were used as control tissue, because of the nonavailability of healthy Car samples. Samples were stored and processed as previously.

**Electron Paramagnetic Resonance Spectroscopy**

Iron levels were analyzed, nondestructively, by electron paramagnetic resonance (EPR) at 77 K.

**Inductively Coupled Plasma Mass Spectroscopy**

Samples were analyzed for total iron, calcium, zinc, and copper as previously.

**Quantification of Protein-Bound Amino Acids and Oxidation Products**

Tissue samples were hydrolyzed to free amino acids and analyzed by HPLC with UV and fluorescence detection as described previously. Protein levels were quantified on homogenates.

**Statistical Analyses**

Data and bivariate correlation analysis was performed using Xtract (© Dr Vladimir Vacata) and Prism (Version 4.0a for Macintosh, GraphPad Software). Correlations are expressed as Pearson correlation coefficients. Mann–Whitney/1-way ANOVA with Tukey’s post hoc multiple comparison tests were used for metal ion and oxidized amino acid levels. P<0.05 was considered significant.

**Results**

**Donor Characteristics and Artery Classification**

Clinical characteristics and sample numbers are presented in supplemental Table I (available online at http://atvb.ahajournals.org). For the postmortem samples, the donor’s clinical record was reviewed for risk factors and cardiovascular events. Two independent investigators, blinded to clinical status, performed the histological analysis. One hundred seven samples from 32 donors were initially examined; samples with inhomogeneous histomorphological classification, Type 2 diabetes, unknown glycemic status, and elevated fasting glucose levels were excluded. To allow stratification of data, cardiovascular events or symptoms indicative of disease including angina pectoris, dyspnea, myocardial infarction, atherosclerosis, transient ischemic attack, and cerebrovascular ischemic stroke were considered.

**Quantification of Iron Levels in Artery Samples by EPR Spectroscopy**

All lesions gave EPR absorptions at g ~4, characteristic of high-spin, rhombic, mononuclear Fe(III) complexes as previously. This signal, which was not detected in control tubes, is distinct from that of Fe(III) heme proteins which have g ~6. Additional EPR absorptions were detected around g ~2, characteristic of organic radicals or iron sulfur clusters; these signals were not investigated further. The g ~4 signal was quantified by double integration of the signal and comparison with standard curves generated using Fe(III)-desferrioxamine. For the postmortem abdominal samples, iron concentrations increased with lesion severity, with significantly higher levels of iron detected in the advanced AbAo compared to Car lesions (Figure 1A). No data for early AbAo lesions was obtained because of low sample numbers. For the corresponding Car samples, there was no significant difference in the level of EPR-detectable iron between the early and advanced lesions (Figure 1A). The Car endarterectomy (CEA) samples, which were all categorized as advanced lesions, contained significantly more EPR-detectable iron than corresponding Car postmortem samples. The concentrations detected in these lesions are similar to those reported previously, with these values being significantly elevated compared to those from healthy artery tissue (Figure 1A).

**Quantification of Metal Ion Levels in Artery Samples by Inductively Coupled Plasma Mass Spectroscopy**

Total lesion iron, copper, calcium and zinc concentrations were determined by inductively coupled plasma mass spectrometry (ICPMS; Figure 1). The iron levels were significantly higher than those detected by EPR, as expected, but the trends were similar. There was an increase in iron levels with lesion severity in the abdominal samples, and high levels
of iron were also detected in the advanced Car specimens. The Car endarterectomy samples contained significantly more iron than the corresponding postmortem Car initial ($P=0.0017$) or advanced ($P=0.024$) lesions.

Zinc and calcium concentrations followed a similar pattern to that detected for iron, with higher levels detected in the more advanced lesions, irrespective of AbAo or Car origin. The levels in the Car endarterectomy samples were higher than the postmortem samples (Figure 1B). Copper concentrations did not vary significantly between the various Car specimens (Figure 1C), and the levels of this metal ion were considerably lower than for iron. Calcium levels were significantly elevated ($P=0.007$) in the Car endarterectomy samples compared to the postmortem initial Car samples. With the exception of copper, the levels of iron, calcium, and zinc measured in the healthy artery samples and initial, preatherosclerotic, Car lesions were similar, and lower than in their advanced counterparts. The detected metal ion concentrations did not correlate with age of the tissue donor (data not shown).

Quantification of Protein Oxidation Products in Artery Samples
We have previously reported elevated levels of protein side-chain oxidation products in advanced Car endarterectomy samples compared to healthy tissue. For the postmortem Car samples, no significant differences were detected between early and advanced lesions for the 4 protein oxida-
Correlation Between Zinc Levels and Protein Oxidation Parameters in Lesions

For the AbAo samples no significant correlation was detected between zinc and any of the protein oxidation markers examined (Table 1). The levels of protein-bound o-Tyr detected in the Car endarterectomy samples were significantly greater than in the initial and advanced postmortem Car samples \( (P = 0.002 \text{ and } 0.0142, \text{ respectively}) \), and m-Tyr levels were significantly elevated in the endarterectomy versus the initial postmortem Car samples \( (P = 0.002 \text{ and } 0.0142, \text{ respectively}) \), and DOPA was not correlated significantly with either EPR-detectable iron \( (r = 0.105, P = 0.25) \) or total iron levels \( (r = 0.161, P = 0.25) \) (data not shown). In contrast, highly-significant positive correlations were detected between zinc and copper concentrations \( (r = 0.891, P = 0.005) \) and calcium \( (r = 0.899, P = 0.0005; \text{ Figure 2 A1 and B1, respectively}) \). A similar pattern was observed with the postmortem Car lesions, with a significant correlation with copper \( (r = 0.438, P = 0.02, \text{ Figure 2 A2}) \), a highly-significant positive correlation detected between zinc and copper concentrations \( (r = 0.891, P = 0.005; \text{ Figure 2 A1 and B1, respectively}) \), but no correlation with either EPR-detectable iron \( (r = 0.129, P = 0.20) \) or total iron levels \( (r = 0.136, P = 0.25) \) (data not shown). The Car endarterectomy samples showed a similar pattern, with significant correlations between zinc and calcium \( (r = 0.895, P = 0.0001; \text{ Figure 2 A3}) \), zinc and copper levels \( (r = 0.363, P = 0.015; \text{ Figure 2 A3}) \), no correlation with total iron levels \( (r = 0.2173, P = 0.154; \text{ data not shown}) \), but a positive correlation between zinc with EPR-detectable iron \( (r = 0.468, P = 0.0013; \text{ data not shown}) \).

Correlation Between Zinc Levels and Other Metal Ions in Lesions

For the postmortem AbAo samples the zinc levels did not correlate significantly with either EPR-detectable iron \( (r = 0.105, P = 0.25) \) or total iron levels \( (r = 0.161, P = 0.25) \) (data not shown). In contrast, highly-significant positive correlations were detected between zinc and copper concentrations \( (r = 0.891, P = 0.005) \) and calcium \( (r = 0.899, P = 0.0005; \text{ Figure 2 A1 and B1, respectively}) \). A similar pattern was observed with the postmortem Car lesions, with a significant correlation with copper \( (r = 0.438, P = 0.02, \text{ Figure 2 A2}) \), a highly-significant positive correlation detected between zinc and copper concentrations \( (r = 0.891, P = 0.005; \text{ Figure 2 A1 and B1, respectively}) \), but no correlation with either EPR-detectable iron \( (r = 0.129, P = 0.20) \) or total iron levels \( (r = 0.136, P = 0.25) \) (data not shown). The Car endarterectomy samples showed a similar pattern, with significant correlations between zinc and calcium \( (r = 0.895, P = 0.0001; \text{ Figure 2 A3}) \), zinc and copper levels \( (r = 0.363, P = 0.015; \text{ Figure 2 A3}) \), no correlation with total iron levels \( (r = 0.2173, P = 0.154; \text{ data not shown}) \), but a positive correlation between zinc with EPR-detectable iron \( (r = 0.468, P = 0.0013; \text{ data not shown}) \).

Correlation Between Zinc Levels and Protein Oxidation Levels in Lesions Stemming From Postmortem Donors With Diagnosed Heart Disease

The relationship between zinc, protein oxidation, and other metal ions was explored further in 2 populations within the postmortem samples groups: those obtained from subjects that died after an infarction or stroke (9 AbAo, 5 Car samples) and a larger group, including the above, obtained from subjects who died from, or who had been diagnosed with, cardiovascular disease (dyspnea, angina pectoris, atherosclerosis; 18 AbAo and 14 Car). Analysis of the data from the first group resulted in positive correlations for the AbAo lesions between zinc and di-Tyr \( (r = 0.91, P = 0.0005) \) and zinc and copper \( (r = 0.512, P = 0.01) \), but all other correlations were insignificant including those between zinc and each measure of iron and between zinc and each marker of protein oxidation. The Car samples were not analyzed because of low numbers.

A similar pattern was detected with the second group of samples. No correlations were detected between zinc and either measure of iron in the AbAo lesions (EPR data, Figure 3 A1; ICPMS data not shown), nor for zinc and any of the markers of protein oxidation. In contrast for the Car lesions a significant negative correlation was detected between zinc and EPR-detectable iron \( (r = 0.664, P = 0.002, \text{Figure 3 A2}) \), but not for total iron levels. In both the AbAo and Car lesions significant correlations were detected between zinc and calcium \( (r = 0.908, P = 0.0005; \text{ Figure 3 B1 and B2}) \) and zinc and copper \( (r = 0.679, P = 0.0025; r = 0.511, P = 0.05 \text{ respectively; Figure 3 C1 and C2}) \).

The similarity between the correlations observed with these samples from subjects with diagnosed cardiovascular disease, and the entire cohort suggests that the clinical background of the subjects (and particularly previous treatment for the diagnosed condition) does not modify the relationship between zinc and the other parameters measured.

### Table 1.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>n</th>
<th>DOPA (μmol/mol Tyr)</th>
<th>di-Tyr (μmol/mol Tyr)</th>
<th>m-Tyr (μmol/mol Phe)</th>
<th>o-Tyr (μmol/mol Phe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Car and AbAo all</td>
<td>53</td>
<td>801±302</td>
<td>43±45</td>
<td>193±125</td>
<td>394±267</td>
</tr>
<tr>
<td>ADV AbAo all</td>
<td>18</td>
<td>865±309</td>
<td>52±48</td>
<td>185±119</td>
<td>427±262</td>
</tr>
<tr>
<td>ADV Car all</td>
<td>6</td>
<td>670±164</td>
<td>14±7</td>
<td>215±146</td>
<td>152±84</td>
</tr>
<tr>
<td>INI Car all</td>
<td>11</td>
<td>948±779</td>
<td>16±7</td>
<td>107±65</td>
<td>224±199</td>
</tr>
<tr>
<td>ADV AbAo male</td>
<td>5</td>
<td>824±360</td>
<td>63±53</td>
<td>172±65</td>
<td>376±197</td>
</tr>
<tr>
<td>ADV AbAo female</td>
<td>13</td>
<td>182±302</td>
<td>26±19</td>
<td>191±127</td>
<td>512±281</td>
</tr>
<tr>
<td>INI Car male</td>
<td>6</td>
<td>748±243</td>
<td>12±4</td>
<td>99±81</td>
<td>131±121</td>
</tr>
<tr>
<td>INI Car female</td>
<td>5</td>
<td>1187±1145</td>
<td>22±8</td>
<td>117±47</td>
<td>337±228</td>
</tr>
<tr>
<td>CEA</td>
<td>44</td>
<td>2102±1092</td>
<td>467±1184</td>
<td>636±641</td>
<td>1130±834</td>
</tr>
<tr>
<td>Healthy</td>
<td>5</td>
<td>1290±567</td>
<td>45±7</td>
<td>679±266</td>
<td>671±337</td>
</tr>
</tbody>
</table>
Discussion
Previous studies have reported positive and negative data on the protective effect of zinc against atherosclerosis.\textsuperscript{17,34,35} It has been proposed that zinc displaces iron and copper from oxidation-vulnerable sites thereby limiting damage.\textsuperscript{9–11} Dietary zinc supplementation in cholesterol-fed rabbits decreases the extent of lesion lipid oxidation and attenuates atherosclerotic burden, despite insignificant changes in lesion zinc.\textsuperscript{19–21} Zinc deficiency in rats has been reported to enhance LDL oxidation in vitro\textsuperscript{36} and in LDL-receptor deficient mice to result in increases in plasma lipids and induction of proinflammatory events.\textsuperscript{37} However zinc supplementation of apoE-deficient mice on a high-fat high-cholesterol diet did not confer protection,\textsuperscript{38} although a hypolipidemic effect was detected, the activity of the antioxidant enzyme superoxide dismutase was elevated, and plasma oxidation was inhibited.\textsuperscript{38} In contrast, zinc may enhance atherosclerosis via increased oxidant generation and decreased high-density lipoprotein levels.\textsuperscript{17,18} Zinc may also modulate atherosclerosis, independent of oxidation, through effects on gene stabilization, transcription factor levels, and apoptosis.\textsuperscript{23,24,26,27}

In the present study we demonstrate that elevated levels of both zinc and iron are present in advanced Car and AbAo lesions, compared to healthy tissue. The iron levels did not

Table 2.

<table>
<thead>
<tr>
<th>Product</th>
<th>CEA</th>
<th>ADV AbAo</th>
<th>ADV Car</th>
<th>INI Car</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPA</td>
<td>(0.2215; P&gt;0.05)</td>
<td>(0.082; P&gt;0.05)</td>
<td>(-0.877; P=0.25)</td>
<td>(-0.655; P=0.02)</td>
</tr>
<tr>
<td>di-Tyr</td>
<td>(-0.077; P&gt;0.05)</td>
<td>(-0.319; P&gt;0.05)</td>
<td>(0.131; P&gt;0.05)</td>
<td>(-0.711; P=0.0143)</td>
</tr>
<tr>
<td>m-Tyr</td>
<td>(0.216; P&gt;0.05)</td>
<td>(0.185; P&gt;0.05)</td>
<td>(0.802; P=0.1)</td>
<td>(-0.166; P=0.25)</td>
</tr>
<tr>
<td>o-Tyr</td>
<td>(0.00086; P&gt;0.05)</td>
<td>(-0.031; P&gt;0.05)</td>
<td>(0.493; P=0.2)</td>
<td>(-0.773; P=0.0053)</td>
</tr>
</tbody>
</table>
correlate inversely with zinc, as would be expected if zinc
replaced iron, with the exception of EPR-detectable iron in
the advanced Car postmortem samples from patients who had
died of cardiovascular events. This negative correlation may
reflect an alteration in the type of iron present in these lesions,
as the total iron levels in these lesions did not correlate with
zinc levels. Copper levels did not vary significantly, and no
inverse correlation was detected between copper and zinc
concentrations; in contrast a positive association was ob-
served. This may be associated with an elevated level of
multiple proteins that bind copper or zinc, or enhanced
expression of (one or more) proteins that contain both metals (eg, the cytosolic and extracellular forms of Cu/Zn superox-
ide dismutase). None of the 4 independent markers of protein
oxidation examined correlated inversely with zinc levels. Previous
studies have shown that iron levels and the extent of protein
oxidation correlate in a positive and significant manner; this
observation has been confirmed here. Because of the
restricted number of samples and sample size, other protein
oxidation markers (eg, tyrosine nitration and thiol oxidation),
or their association with zinc, were assessed. However, the
lack of correlation between zinc and the 4 independent
markers of protein oxidation examined suggests that zinc is
unlikely to protect against transition-metal induced protein
oxidation.

In contrast, highly significant correlations were detected
between zinc and calcium in all lesions. These metal ions
bind to similar ligands in vitro. These data are consistent
with an increased availability of metal ion binding sites,
potentially including polyanionic glycosaminoglycans and
proteoglycans, which have a high affinity for metal ions.
It is unclear whether calcium accumulation occurs concur-
rently with zinc, but the strength of the observed correlations
supports this conclusion. It is not possible to ascertain
whether calcium and zinc accumulation occurs independently
of iron and copper, or whether all of these metal ions
accumulate concurrently. Little is known about the require-
ments and functions of zinc in maintaining the integrity of the
vasculature and the vascular endothelium. Modifications in
zinc homeostasis may result in changes in the cellular labile
zinc pool and subsequent modulation of the function and
activity of zinc-requiring proteins and signaling pathways, in
dermal cells. As the number of zinc-binding proteins is
large, determining which proteins are responsible will be

Figure 3. Correlation plots of zinc vs EPR-
detectable iron (A1, A2), calcium (B1, B2), and
copper (C1, C2) from postmortem abdominal
aorta (A1, B1, C1) and carotid artery samples
(A2, B2, C2) from subjects with diagnosed card-
iovascular disease. Correlation coefficients (r)
and probability values are as indicated.
onerous. A limitation of this study is that only total levels of the metal ions were quantified, rather than bioavailable levels, and that the lesions examined are heterogenous in nature.

The data obtained provide a potential explanation for the reported protective effect of elevated zinc against atherosclerosis. It is well established that highly-fibrotic calcified lesions are less prone to rupture than lipid-rich, matrix-poor, le-

45 The decreased extent of cardiovascular events in people with high zinc levels may therefore merely be an indicator of calcium accumulation and fibrosis and hence decreased propensity to lesion rupture. Whether high zinc levels are merely a consequence of calcification and the presence of fibrous lesions or can promote the formation of stable lesions is unclear. Thus high zinc levels may promote lesion stability by binding to matrix components and stabilizing lesion structures; this hypothesis would appear to be worthy of further study. If correct, it may offer novel mechanisms of enhancing lesion stability.

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Disclosures
None.

References

2. Stocker R, Keeney JE. Jr. Role of oxidative modifications in atheroscler-


3. Stanley N, Stadler N, Woods AA, Bannon PG, Davies MJ. Concentra-


5. Danesh J, Appleby P. Coronary heart disease and iron status: meta-anal-


10. Wilkins GM, Leake DS. The oxidation of low density lipoprotein by cells or iron is inhibited by zinc. F E B S L e t t . 1994;341:259–262.


13. Singh RB, Gupta UC, Mittal N, Niaz MA, Ghosh S, Rastogi V. Epide-


19. Alissa EM, Bahjiri SM, Lamb DJ, Ferns GA. The effects of coadminis-

tration of dietary copper and zinc supplements on atherosclerosis, anti-


20. Ren M, Rajendran R, Ning P, Tan Kwong Huat B, Choon Nam O, Watt F, Jenner A, Halliwell B. Zinc supplementation decreases the develop-


24. Reither G, Toborek M, Hennig B. Peroxisome proliferator activated receptor alpha and gamma require zinc for their anti-inflammatory prop-


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### Supplementary Table I.

Characteristics of lesion samples examined: sample numbers and sample stratification according to gender, artery type, lesion type and clinical characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Abdominal aorta samples</th>
<th>Carotid artery samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of donors (yrs)</td>
<td>77.7 ± 10.9</td>
<td>74.9 ± 12.3</td>
</tr>
<tr>
<td>Samples numbers and gender</td>
<td>27 (16 female, 11 male)</td>
<td>26 (11 female, 15 male)</td>
</tr>
<tr>
<td>Donors with myocardial infarction as death cause</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Donors with diagnosed heart disease</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Smokers</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Advanced lesions</td>
<td>19</td>
<td>6</td>
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<tr>
<td>Intermediate lesions</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Preatherosclerotic, initial lesions</td>
<td>-</td>
<td>11</td>
</tr>
<tr>
<td>Endarterectomy samples</td>
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<td>44</td>
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