Perivascular Adipose Adiponectin Correlates With Symptom Status of Patients Undergoing Carotid Endarterectomy

Gaurav Sharma, MD; Ming Tao, MD; Kui Ding, MD; David Yu, BS; William King; Galina Deyneko, MD; Xiaosong Wang, MD; Alban Longchamp, MD; Frederick J. Schoen, MD, PhD; C. Keith Ozaki, MD; Marcus E. Semel, MD, MPH

**Background and Purpose**—Recent symptoms stand as a major determinant of stroke risk in patients with carotid stenosis, likely reflective of atherosclerotic plaque destabilization. In view of emerging links between vascular and adipose biology, we hypothesized that human perivascular adipose characteristics associate with carotid disease symptom status.

**Methods**—Clinical history, carotid plaques, blood, and subcutaneous and perivascular adipose tissues were prospectively collected from patients undergoing carotid endarterectomy. Nine adipose-associated biological mediators were assayed and compared in patients with symptomatic (n=15) versus asymptomatic (n=19) disease. Bonferroni correction was performed for multiple testing (α/9=0.006).

**Results**—Symptomatic patients had 1.9-fold higher perivascular adiponectin levels ($P=0.005$). Other circulating, subcutaneous, and perivascular biomarkers, as well as microscopic plaque characteristics, did not differ between symptomatic and asymptomatic patients.

**Conclusions**—Symptomatic and asymptomatic carotid endarterectomy patients display a tissue-specific difference in perivascular adipose adiponectin. This difference, which was not seen in plasma or subcutaneous compartments, supports a potential local paracrine relationship with vascular disease processes that may be related to stroke mechanisms.

(Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.114.008468.)

**Key Words:** adipokines ■ adiponectin ■ atherosclerosis ■ carotid stenosis ■ endarterectomy, carotid

Symptomatic status in carotid stenosis confers a 26% 2-year stroke risk without surgical intervention. Plaque destabilization is thought to account for these differences when degrees of stenosis are comparable; however, underlying mechanisms are not well understood.

Perivascular adipose tissue is increasingly recognized for its active role in cell–cell signaling, modulation of smooth muscle function, remodeling, and inflammation. In mice, transplantation of visceral adipose tissue to the carotid leads to impaired endothelial function and atherogenesis. Circulating plasma adiponokine levels correlate with carotid intima-media thickness and symptom status in humans.

Theorizing complex signaling interplay among subcutaneous/perivascular adipose tissues and the adjacent carotid, we investigated our hypothesis that specific adipose–related biomarkers would uniquely link to clinical features of carotid endarterectomy (CEA) patients.

**Methods**

**Study Participants and Data Collection**

Patients undergoing CEA for symptomatic or asymptomatic carotid stenosis at a single institution in 2013 provided written informed consent for prospective collection of demographic, clinical, and duplex ultrasonography data under a Partners Human Research Committee institutional review board–approved protocol. All patients underwent conventional CEA via a longitudinal arteriotomy.

**Sample Procurement and Protein Assay**

At the time of surgery, peripheral blood, subcutaneous, and perivascular tissues were harvested. After protein isolation as previously described, adiponectin, interleukin (IL)-1β, IL-6, IL-8, leptin, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, resistin, and tumor necrosis factor were quantified via Luminex multiplex assay (Methods in the online-only Data Supplement).

**Histology**

Random plaque sections were prepared by conventional histological methods and stained with hematoxylin/eosin, Masson trichrome, and immunohistochemical anti-CD68 staining (Ventana Medical Systems, Inc, Tucson, AZ). Fibrous cap, necrotic core, angio genesis, macrophage content, plaque hemorrhage, and calcification were semiquantitatively assessed by a blinded vascular pathologist.

**Statistical Analysis**

Categorical variables were compared using Fisher exact testing. Continuous data were analyzed using Wilcoxon signed-rank test or
the Student t test based on the normality of distribution. Bonferroni correction was used for multiple comparisons. All statistical analyses were conducted using SAS software, version 9.3 (SAS Institute, Inc, Cary, NC).

Results

Nineteen patients with asymptomatic carotid stenosis and 15 patients with symptomatic disease were enrolled. Both groups were similar in terms of baseline characteristics, including previous transient ischemic attack/stroke unrelated to the current carotid lesion, antiplatelet therapy, statin use, and carotid artery peak systolic velocity. CEA represented primary intervention in all patients, none of whom had had previous cervical radiation therapy. Symptomatic patients differed significantly after Bonferroni adjustment (r=−0.38; P=0.03), which was not significant after Bonferroni correction, as well as a significant positive correlation between lepition and body mass index (r=0.59; P=0.0003).

Comparison of symptomatic versus asymptomatic patients revealed that the former had 1.9-fold higher perivascular adiponectin (P=0.005; Figure 1; Table I in the online-only Data Supplement). This association was robust, remaining statistically significant after Bonferroni adjustment (α/9=0.006). Symptomatic patients demonstrated trends toward (1) plasma IL-1β decrease (2.7-fold; P=0.008), (2) subcutaneous adiponectin elevation (1.5-fold; P=0.04), and plasminogen activator inhibitor-1 decrease (2-fold; P=0.01), and (3) perivascular IL-1β elevation (1.4-fold; P=0.015; Tables I–III in the online-only Data Supplement).

Endarterectomy specimen pathological scoring did not differ between symptomatic and asymptomatic patients (Figure 2; Table IV in the online-only Data Supplement). There were no significant differences between perivascular mediator levels and histological characteristics.

Discussion

Here, we find that perivascular adiponectin expression differs based on symptom status in patients undergoing CEA. This differential expression profile was only present in the perivascular compartment—symptomatic patients had similar circulating adiponectin levels when compared with their asymptomatic counterparts but had significantly higher adiponectin expression in tissue contiguous to the carotid artery. There were several other adipose-related biomarker trends between these small cohorts, further supporting potential signaling pathways between the vasculature and these tissues.

Little data exist on perivascular adiponectin in humans. In a previous report, we did note decreased perivascular adiponectin compared with the subcutaneous compartment in major leg amputation specimens. Decreased plasma adiponectin levels have been found to be associated with increased coronary artery atherogenesis, plaque vulnerability, and carotid intima-media thickness. Here, a seemingly paradoxical relationship was found between local perivascular adiponectin (traditionally viewed as a protective vascular mediator) and symptom status (a clinical marker of destabilized plaque).

This may suggest disparate roles for local versus systemic adiponectin or undefined effects of adiponectin.

In this cohort, pathological characteristics of carotid plaques were similar between symptomatic and asymptomatic patients. The observation that perivascular expression of adiponectin was significantly associated with symptom status

<table>
<thead>
<tr>
<th>Table. Baseline Patient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic, n=19</td>
</tr>
<tr>
<td>Mean age (SD)</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>White (%)</td>
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<tr>
<td>BMI (SD)</td>
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<tr>
<td>Comorbidities</td>
</tr>
<tr>
<td>Remote TIA/stroke (%)</td>
</tr>
<tr>
<td>Peripheral arterial disease (%)</td>
</tr>
<tr>
<td>Previous myocardial infarction (%)</td>
</tr>
<tr>
<td>Previous coronary intervention (%)</td>
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<tr>
<td>Heart failure (%)</td>
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<tr>
<td>Hypertension (%)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
</tr>
<tr>
<td>Renal insufficiency, Cr ≥ 2 (%)</td>
</tr>
<tr>
<td>Smoking status</td>
</tr>
<tr>
<td>Never (%)</td>
</tr>
<tr>
<td>Current (%)</td>
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<tr>
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<td>Aspirin (%)</td>
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<td>Clopidogrel (%)</td>
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<td>Warfarin (%)</td>
</tr>
<tr>
<td>Low molecular weight heparin (%)</td>
</tr>
<tr>
<td>Preoperative internal carotid stenosis</td>
</tr>
<tr>
<td>Mean PSV, cm/s (SD)</td>
</tr>
<tr>
<td>Degree of internal carotid stenosis</td>
</tr>
<tr>
<td>&lt;49% (%)</td>
</tr>
<tr>
<td>50% to 69% (%)</td>
</tr>
<tr>
<td>70% to 99% (%)</td>
</tr>
<tr>
<td>Carotid disease symptomatology</td>
</tr>
<tr>
<td>Stroke (%)</td>
</tr>
<tr>
<td>Hemispheric TIA (%)</td>
</tr>
<tr>
<td>Amusia/anosognosia (%)</td>
</tr>
<tr>
<td>Days from symptoms to CEA (IQR)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; BMI, body mass index; CEA, carotid endarterectomy; Cr, creatinine; IQR, interquartile range; PSV, peak systolic velocity; and TIA, transient ischemic attack.

*P value obtained by Student t test based on the normal distribution. Others were obtained by nonparametric testing.
whereas plaque histology was not supported that the former association has a larger effect size. Note that carotid plaques are complex, and the microscopic analyses may have sampling errors. Alternatively, local adiponectin expression and symptom development may be linked via plaque destabilization–independent mechanisms.

Limitations are acknowledged. In total, we obtained single-perivascular adipose samples from 34 patients. Assuming a type I error rate \( \alpha \) equals to 0.05 and type II error rate \( \beta \) equals to 0.10 (90% power), our study is powered to detect effect sizes of 0.6; subtle differences in protein levels can be missed, and the interesting trends noted in the other mediators may be biologically noteworthy. Type I error (eg, in the association seen between symptom status and perivascular adiponectin) was addressed by Bonferroni correction. Nearly all of our patients were whites, which may limit the generalizability of our results. The duration of plaque-stabilizing medication use and compliance in each group were not clear from our source data. The current piece examines beyond the vessel wall to the surrounding adipose; thus, detailed plaque morphology (complete plaque sectioning with search for intraplaque hemorrhage) was not the focus of the study because knowledge relating plaque characteristics and symptoms is widely accepted. We do include some basic plaque morphology data because it may relate to the adipose phenotype. Most importantly, precise mechanisms cannot be derived from this observational data, although the novel associations discovered should spur such important studies.

**Conclusions**

Symptomatic CEA patients exhibit 2-fold higher local, perivascular adipose adiponectin levels. This novel link suggests previously unrecognized relationships among the neurological, vascular, and adipose organs, and it may lead to strategies to improve patient selection and other measures for stroke risk reduction.

**Sources of Funding**

This research was generously supported by National Institutes of Health 5T32CA009535-26, 5T35HL110843, American Heart Association 12GRNT9510001/12GRNT1207025, Swiss National Science Foundation P1LAP3_158888, the Lea Carpenter du Pont Vascular Surgery Fund, and the Carl and Ruth Shapiro Family Foundation.

**Disclosures**

This investigator-initiated work represents a joint research venture between Brigham and Women’s Hospital and Novartis Institutes, who provided a portion of the research expenses. Dr Ozaki was also supported by the American Heart Association Grant-in-Aid.

**References**


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Stroke. published online May 12, 2015;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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http://stroke.ahajournals.org/content/early/2015/05/12/STROKEAHA.114.008468

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2015/05/12/STROKEAHA.114.008468.DC1

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Supplemental Material

Perivascular Adipose Adiponectin Correlates with Symptom Status of Patients Undergoing Carotid Endarterectomy
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1. Department of Surgery, Brigham and Women’s Hospital/Harvard Medical School, Boston, MA; 2. University of Washington School of Medicine, Seattle, WA; 3. Novartis Institutes for BioMedical Research, Cambridge, MA; 4. Department of Pathology, Brigham and Women’s Hospital/Harvard Medical School, Boston, MA

Supplemental Methods

Methods for protein assay from subcutaneous and perivascular adipose tissues have been described previously. 1 At the time of surgery, peripheral blood, subcutaneous adipose tissue (at the site of the cervical incision), and perivascular adipose tissue (contiguous to the adventitia of the diseased common and internal carotid arteries) of patients undergoing carotid endarterectomy (CEA) were obtained by six participating surgeons who had been briefed on the standardized sampling techniques. Fifteen milliliters of peripheral blood were obtained at the time of peripheral intravenous line placement and plasma was isolated by centrifugation for 15 minutes at 2000g at room temperature. Surgeons performing the carotid endarterectomy collected 50 to 500 milligrams of adipose tissue from each of two locations: (1) subcutaneous tissue at the site of neck incision, and (2) perivascular tissue contiguous to the adventitia of diseased segments of the operative carotid artery. All samples were immediately flash frozen in liquid nitrogen then stored at -80°C until the time of analysis. Proteins were isolated from the samples using ice-cold Dulbecco’s phosphate-buffered saline with protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN). This solution was then homogenized and centrifuged (2,000g x 5 minutes) to remove gross debris. Homogenates were again centrifuged (10,000g x 10 minutes). The supernatant was then collected for quantitative protein analysis using a Luminex multiple antigen flow microparticle bead assay (Luminex Corporation, Austin, TX). Based on previous literature, nine key biologic mediators were assayed: adiponectin, interleukin (IL)-1β, IL-6, IL-8, leptin, monocyte chemoattractant protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, resistin, and tumor necrosis factor (TNF). 2-20 Quantities were adjusted by the total volume of each sample.
**Supplemental Tables and Figures**

**Supplemental table I.** Perivascular adipose tissue protein levels in patients with asymptomatic and symptomatic carotid artery stenosis.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Asymptomatic (n=19)</th>
<th>Symptomatic (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, pg/ml</td>
<td>251908.70</td>
<td>478445.50</td>
<td>0.005</td>
</tr>
<tr>
<td>[Q1,Q3]</td>
<td>[212749.00,365392.00]</td>
<td>[315447.00,603897.00]</td>
<td></td>
</tr>
<tr>
<td>IL-1β, pg/ml [Q1,Q3]</td>
<td>0.33 [0.19,0.42]</td>
<td>0.47 [0.33,0.77]</td>
<td>0.015</td>
</tr>
<tr>
<td>IL-6, pg/ml [Q1,Q3]</td>
<td>8.56 [2.02,35.06]</td>
<td>14.39 [4.45,33.91]</td>
<td>0.68</td>
</tr>
<tr>
<td>IL-8, pg/ml [Q1,Q3]</td>
<td>9.52 [3.57,25.03]</td>
<td>6.92 [4.63,25.12]</td>
<td>0.81</td>
</tr>
<tr>
<td>Leptin, pg/ml [Q1,Q3]</td>
<td>[353.70,1009.52]</td>
<td>549.96 [376.10,940.69]</td>
<td>0.75</td>
</tr>
<tr>
<td>MCP-1, pg/ml [Q1,Q3]</td>
<td>146.31 [76.48,331.81]</td>
<td>170.33 [99.87,369.96]</td>
<td>0.47</td>
</tr>
<tr>
<td>PAI-1, pg/ml [Q1,Q3]</td>
<td>193.28 [97.40,564.58]</td>
<td>235.23 [102.73,327.21]</td>
<td>0.45</td>
</tr>
<tr>
<td>Resistin, pg/ml [Q1,Q3]</td>
<td>22351.51</td>
<td>2479.73</td>
<td></td>
</tr>
<tr>
<td>TNF, pg/ml [Q1,Q3]</td>
<td>0.91 [0.75,1.30]</td>
<td>0.96 [0.78, 3.83]</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Q1, 25% quartile; Q3, 75% quartile; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor

**Supplemental table II.** Plasma protein levels in patients with asymptomatic and symptomatic carotid artery stenosis.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Asymptomatic (n=19)</th>
<th>Symptomatic (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, pg/ml</td>
<td>1045239</td>
<td>10845269</td>
<td>0.31</td>
</tr>
<tr>
<td>[Q1,Q3]</td>
<td>[6685570,12945900]</td>
<td>[6792411,23297723]</td>
<td></td>
</tr>
<tr>
<td>IL-1β, pg/ml [Q1,Q3]</td>
<td>0.43 [0.27,1.0]</td>
<td>0.16 [0.00, 0.34]</td>
<td>0.008</td>
</tr>
<tr>
<td>IL-6, pg/ml [Q1,Q3]</td>
<td>4.38 [2.6,9.99]</td>
<td>3.24 [1.7,5.63]</td>
<td>0.17</td>
</tr>
<tr>
<td>IL-8, pg/ml [Q1,Q3]</td>
<td>5.07 [4.3,4.91]</td>
<td>4.68 [4.0,6.39]</td>
<td>0.45</td>
</tr>
<tr>
<td>Leptin, pg/ml [Q1,Q3]</td>
<td>12322.57,26225.14</td>
<td>5147.19,20155.25</td>
<td>0.77</td>
</tr>
<tr>
<td>MCP-1, pg/ml [Q1,Q3]</td>
<td>18861.77</td>
<td>10839.31</td>
<td></td>
</tr>
<tr>
<td>PAI-1, pg/ml [Q1,Q3]</td>
<td>391.73,205.39</td>
<td>104.43 [92.05,144.83]</td>
<td>0.39</td>
</tr>
<tr>
<td>Resistin, pg/ml [Q1,Q3]</td>
<td>24020.40</td>
<td>24994.83</td>
<td></td>
</tr>
<tr>
<td>TNF, pg/ml [Q1,Q3]</td>
<td>3.26 [2.82,5.81]</td>
<td>3.32 [2.64,4.95]</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Q1, 25% quartile; Q3, 75% quartile; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor

**Supplemental table III.** Subcutaneous adipose tissue protein levels in patients with asymptomatic and symptomatic carotid artery stenosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Asymptomatic (n=19)</th>
<th>Symptomatic (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, pg/ml [Q1,Q3]</td>
<td>428061.10 [251057.00,1065337.00]</td>
<td>661021.00 [551276.00,1226455.00]</td>
<td>0.04</td>
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<tr>
<td>IL-1β, pg/ml [Q1,Q3]</td>
<td>0.41 [0.31,0.54]</td>
<td>0.45 [0.37,0.48]</td>
<td>0.35</td>
</tr>
<tr>
<td>IL-6, pg/ml [Q1,Q3]</td>
<td>2.15 [1.70,22.50]</td>
<td>2.61 [1.82,7.68]</td>
<td>0.81</td>
</tr>
<tr>
<td>Leptin, pg/ml [Q1,Q3]</td>
<td>513.41 [329.96,725.18]</td>
<td>544.96 [234.01,1069.47]</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>MCP-1, pg/ml [Q1,Q3]</td>
<td>147.54 [64.35,171.86]</td>
<td>95.12 [54.18,168.89]</td>
<td>0.30</td>
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<tr>
<td>PAI-1, pg/ml [Q1,Q3]</td>
<td>193.66 [142.32,281.30]</td>
<td>96.81 [62.46,185.27]</td>
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<tr>
<td>Resistin, pg/ml [Q1,Q3]</td>
<td>17291.50 [11911.40,34623.52]</td>
<td>16271.41 [13223.43,38649.57]</td>
<td>0.92</td>
</tr>
<tr>
<td>TNF, pg/ml [Q1,Q3]</td>
<td>0.22 [0.17,0.32]</td>
<td>0.25 [0.16,0.34]</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Q1, 25% quartile; Q3, 75% quartile; IL-1β, interleukin-1 beta; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF, tumor necrosis factor

**Supplemental table IV.** Univariate analysis of pathologic characteristics of carotid endarterectomy plaques and symptomatic status.

<table>
<thead>
<tr>
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<th>Asymptomatic (n=19)</th>
<th>Symptomatic (n=15)</th>
<th>P value</th>
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<td>Necrotic core</td>
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<td>3</td>
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<tr>
<td></td>
<td>&lt;15%</td>
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<td></td>
<td>15-30%</td>
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<td></td>
<td>&gt;30%</td>
<td>10</td>
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<tr>
<td>Lesion cap</td>
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<td>7</td>
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<td></td>
<td>No</td>
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<tr>
<td>Revascularization</td>
<td>Yes</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11</td>
<td>10</td>
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<td>---------------------------</td>
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<tr>
<td>Acute plaque hemorrhage</td>
<td>(- )</td>
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<td>14</td>
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<tr>
<td></td>
<td>(+/-)</td>
<td>1</td>
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<tr>
<td></td>
<td>Complicated atheroma</td>
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**Supplemental References**


