

## **SUPPLEMENTAL MATERIAL**

### **Perivascular Adipose Adiponectin Correlates with Symptom Status of Patients Undergoing Carotid Endarterectomy**

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### **Supplemental Methods**

Methods for protein assay from subcutaneous and perivascular adipose tissues have been described previously.<sup>1</sup> 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)-1 $\beta$ , IL-6, IL-8, leptin, monocyte chemoattractant protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, resistin, and tumor necrosis factor (TNF).<sup>2-20</sup> 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.

| <i>Protein</i>               | <i>Asymptomatic (n=19)</i>         | <i>Symptomatic (n=15)</i>          | <i>P-value</i> |
|------------------------------|------------------------------------|------------------------------------|----------------|
| Adiponectin, pg/ml [Q1,Q3]   | 251908.70<br>[212749.00,365392.00] | 478445.50<br>[315447.00,603897.00] | <b>0.005</b>   |
| IL-1 $\beta$ , pg/ml [Q1,Q3] | 0.33 [0.19,0.42]                   | 0.47 [0.33,0.77]                   | <b>0.015</b>   |
| IL-6, pg/ml [Q1,Q3]          | 8.56 [2.02,35.06]                  | 14.39 [4.45,33.91]                 | 0.68           |
| IL-8, pg/ml [Q1,Q3]          | 9.52 [3.57,25.03]                  | 6.92 [4.63,25.12]                  | 0.81           |
| Leptin, pg/ml [Q1,Q3]        | 470.29<br>[353.70,1009.52]         | 549.96 [376.10,940.69]             | 0.75           |
| MCP-1, pg/ml [Q1,Q3]         | 146.31 [76.48,331.81]              | 170.33 [99.87,369.96]              | 0.47           |
| PAI-1, pg/ml [Q1,Q3]         | 193.28 [97.40,564.58]              | 150.23 [102.73,327.21]             | 0.45           |
| Resistin, pg/ml [Q1,Q3]      | 22351.51<br>[11800.63,42900.90]    | 24379.73<br>[12545.36,29878.99]    | 0.78           |
| TNF, pg/ml [Q1,Q3]           | 0.91 [0.75,1.30]                   | 0.96 [0.78,3.83]                   | 0.31           |

*Q1*, 25% quartile; *Q3*, 75% quartile; *IL-1 $\beta$* , 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.

| <i>Protein</i>               | <i>Asymptomatic (n=19)</i>      | <i>Symptomatic (n=15)</i>       | <i>P-value</i> |
|------------------------------|---------------------------------|---------------------------------|----------------|
| Adiponectin,pg/ml [Q1,Q3]    | 10452329<br>[6685570,12945900]  | 10845269<br>[6792411,22397723]  | 0.31           |
| IL-1 $\beta$ , pg/ml [Q1,Q3] | 0.43 [0.27,1.0]                 | 0.16 [0.00, 0.34]               | <b>0.008</b>   |
| IL-6, pg/ml [Q1,Q3]          | 4.38 [2.62,9.99]                | 3.24 [1.17,5.63]                | 0.17           |
| IL-8, pg/ml [Q1,Q3]          | 5.07 [4.34,9.11]                | 4.68 [4.05,6.39]                | 0.45           |
| Leptin, pg/ml [Q1,Q3]        | 18861.77<br>[12322.57,26225.14] | 10839.31<br>[5147.19,20155.25]  | 0.77           |
| MCP-1, pg/ml [Q1,Q3]         | 116.76<br>[91.73,205.39]        | 104.43 [92.05,144.83]           | 0.39           |
| PAI-1, pg/ml [Q1,Q3]         | 24020.40<br>[19035.50,30109.30] | 24994.83<br>[17196.20,27649.40] | 0.68           |
| Resistin, pg/ml [Q1,Q3]      | 29733.15<br>[20704.90,36079.70] | 27822.40<br>[20228.50,32572.90] | >0.99          |
| TNF, pg/ml [Q1,Q3]           | 3.26 [2.82,5.81]                | 3.32 [2.64,4.95]                | 0.92           |

*Q1*, 25% quartile; *Q3*, 75% quartile; *IL-1 $\beta$* , 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.

| <i>Variable</i>              | <i>Asymptomatic (n=19)</i>       | <i>Symptomatic (n=15)</i>        | <i>P-value</i> |
|------------------------------|----------------------------------|----------------------------------|----------------|
| Adiponectin, pg/ml [Q1,Q3]   | 428061.10 [251057.00,1065337.00] | 661021.00 [551276.00,1226455.00] | <b>0.04</b>    |
| IL-1 $\beta$ , pg/ml [Q1,Q3] | 0.41 [0.31,0.45]                 | 0.45 [0.37,0.48]                 | 0.35           |
| IL-6, pg/ml [Q1,Q3]          | 2.15 [1.70,22.50]                | 2.61 [1.82,7.68]                 | 0.81           |
| IL-8, pg/ml [Q1,Q3]          | 9.31 [4.62,23.23]                | 7.15 [3.62,11.16]                | 0.19           |
| Leptin, pg/ml [Q1,Q3]        | 513.41 [329.96,725.18]           | 544.96 [234.01,1069.47]          | >0.99          |
| MCP-1, pg/ml [Q1,Q3]         | 147.54 [64.35,171.86]            | 95.12 [54.18,168.89]             | 0.30           |
| PAI-1, pg/ml [Q1,Q3]         | 193.66 [142.32,281.30]           | 96.81 [62.46,185.27]             | <b>0.01</b>    |
| Resistin, pg/ml [Q1,Q3]      | 17291.50 [11911.40,34623.52]     | 16271.41 [13223.43,38649.57]     | 0.92           |
| TNF, pg/ml [Q1,Q3]           | 0.22 [0.17,0.32]                 | 0.25 [0.16,0.34]                 | 0.88           |

*Q1*, 25% quartile; *Q3*, 75% quartile; *IL-1 $\beta$* , 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.

|                   |        | <i>Asymptomatic (n=19)</i> | <i>Symptomatic (n=15)</i> | <i>P value</i> |
|-------------------|--------|----------------------------|---------------------------|----------------|
| Necrotic core     | None   | 3                          | 2                         | 0.95           |
|                   | <15%   | 2                          | 2                         |                |
|                   | 15-30% | 4                          | 2                         |                |
|                   | >30%   | 10                         | 9                         |                |
| Lesion cap        | Yes    | 7                          | 5                         | >0.99          |
|                   | No     | 12                         | 10                        |                |
| Revascularization | Yes    | 6                          | 5                         | >0.99          |

|                          |                      |    |    |       |
|--------------------------|----------------------|----|----|-------|
|                          | No                   | 11 | 10 |       |
| Acute plaque hemorrhage  | (-)                  | 16 | 14 |       |
|                          | (+/-)                | 1  | 0  | >0.99 |
|                          | (+)                  | 1  | 0  |       |
|                          | (++)                 | 1  | 1  |       |
|                          | Yes                  | 3  | 3  | >0.99 |
| Calcification            | No                   | 16 | 12 |       |
| Macrophage content grade | <15%                 | 1  | 1  |       |
|                          | 15-30%               | 9  | 7  | >0.99 |
|                          | 31-50%               | 7  | 5  |       |
|                          | >50%                 | 2  | 2  |       |
|                          | Intimal thickening   | 0  | 0  |       |
| Atherosclerosis stage    | Early atheroma       | 0  | 0  | >0.99 |
|                          | Fibroatheroma        | 3  | 3  |       |
|                          | Complicated atheroma | 16 | 12 |       |

### Supplemental References

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