Increased expression and secretion of resistin in epicardial adipose tissue of patients with acute coronary syndrome

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ACUTE CORONARY SYNDROME (ACS) represents a complex phenotype involving the interplay between endothelial dysfunction and proinflammatory and prothombotic components under the control of environmental and genetic factors (2). In the last decades, much attention has been paid to inflammatory biomarkers that affect several pathways leading to plaque erosion or rupture and therefore could play a role in ACS (3). High circulating levels of some of these biomarkers, together with their high expression level in coronary or carotid plaques, are prominent features of patients with unstable angina or non-ST elevation myocardial infarction (NSTEMI).

Adipose tissue functions as an endocrine organ that produces and secretes bioactive adipokines with pro- and anti-inflammatory properties that could contribute to the inflammatory activation in ACS. The epicardial adipose tissue (EAT) has been recognized as a source of proinflammatory cytokines. EAT covers the surface of the heart in human and some animal species and has a close functional and anatomic relationship with epicardial coronary arteries and myocytes, thus with possible interaction among these components (13). An augmented inflammatory response associated with considerable macrophage infiltration has been demonstrated in EAT, compared with subcutaneous fat, of patients with significant coronary artery disease (CAD), although without any distinction between chronic and acute manifestations of the disease (4, 5, 20).

Endothelial damage and dysfunction also have an important role in the initiation and progression of atherosclerotic disease, as well as in the development of ACS. Several studies have shown cross-talk between adipose tissue and endothelial cells of nearby vessels. Direct effects of certain adipokines on expression and secretion of adhesion molecules and on endothelial cell permeability have been hypothesized to interact with vasa vasorum, smooth muscle cells, endothelium, and cellular components of the plaque by diffusion in interstitial fluid across adventitia, media, and intima, or to be released from EAT directly in vasa vasorum and transported downstream into the arterial wall (11, 26, 30).

In the present study, we evaluated the expression of several adipokines in EAT of male patients undergoing coronary artery bypass graft (CABG) surgery for ACS or age- and body mass index (BMI)-matched patients with stable CAD. Patients undergoing cardiac surgery for mitral insufficiency, with angiographically normal coronary arteries, served as a control group.

Study population. Sixty-six male patients who underwent elective CABG surgery for ACS (n = 32) or stable CAD (n = 34) and 23 patients who underwent surgery for mitral valve repair (control group) were enrolled in the study. The ACS group was represented by patients with a diagnosis of unstable angina or NSTEMI, whereas the stable CAD group was represented by patients with angina symptoms stable for at least three months, with angiographically documented coronary stenoses (>50%). The control group was characterized by normal coronary arteries on angiography and serum C-reactive protein (CRP) levels <2.5 mg/l. All patients were recruited in the Divisions of Cardiac Surgery, San Raffaele Scientific Institute, Milan, and Ospedali Riuniti, Trieste. Demographic and clinical characteristics of the patients are shown in Table 1. Briefly, information regarding age,
**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stable CAD</th>
<th>ACS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>23</td>
<td>34</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>52.0 ± 2.4</td>
<td>63.4 ± 1.5*</td>
<td>66.0 ± 1.8*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 ± 0.5</td>
<td>26.3 ± 0.5</td>
<td>26.6 ± 0.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>95 ± 3</td>
<td>100 ± 2</td>
<td>101 ± 2</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Risk factors, %**

- Dyslipidemia: 16 vs. 62* vs. 59* P < 0.0001
- Diabetes: 0 vs. 31* vs. 26* P < 0.0001
- Hypertension: 30 vs. 72* vs. 64* P < 0.0001
- Current smoking: 20 vs. 7 vs. 22 P = 0.12
- Family history: 25 vs. 28 vs. 15 P = 0.48

**Medications, %**

- Aspirin: 15 vs. 70* vs. 95* P < 0.0001
- Statin: 5 vs. 63* vs. 68* P < 0.0001
- ACEI/ARB: 45 vs. 60 vs. 59 P = 0.55
- β-Blocker: 30 vs. 77* vs. 86* P = 0.002
- Clopidogrel/ticlopidine: 0 vs. 13 vs. 23* P = 0.03
- Calcium channel blocker: 20 vs. 13 vs. 14 P = 0.80
- Diuretics: 5 vs. 10 vs. 18 P = 0.38
- Nitroglycerin: 0 vs. 37% vs. 77%* P < 0.0001
- Anticoagulants: 10 vs. 3 vs. 9 P = 0.57
- Heparin: 0 vs. 7 vs. 74%* P < 0.0001
- OHAs: 0 vs. 13 vs. 5 P = 0.10
- Insulin: 0 vs. 0 vs. 18%* P = 0.007

**Coronary artery disease, %**

- One vessel: 7 vs. 6 vs. 0.82
- Two vessel: 14 vs. 9
- Three vessel or left main: 79 vs. 84

Values are means ± SE or geometric mean and confidence interval. CAD, coronary artery disease; ACS, acute coronary syndrome; BMI, body mass index; OHAs, oral hypoglycemic agents. ACEI/ARB, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker. ANOVA and post hoc Tukey-Kramer test: P < 0.05 vs. control (*) and stable CAD (†).

**Table 2. Serum measurements**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stable CAD</th>
<th>ACS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>34</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.84 ± 0.07</td>
<td>0.90 ± 0.05</td>
<td>0.94 ± 0.06</td>
<td>0.57</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>97 (85–110)</td>
<td>104 (95–114)</td>
<td>100 (89–113)</td>
<td>0.44</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>3.3 (2.0–5.5)</td>
<td>5.7 (3.9–8.2)</td>
<td>6.2 (3.9–10.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>63 (48–82)</td>
<td>73 (60–88)</td>
<td>87 (68–112)</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL chol, mg/dl</td>
<td>151 ± 10</td>
<td>110 ± 8*</td>
<td>96 ± 6*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>APO A-I, mg/dl</td>
<td>44 ± 3</td>
<td>37 ± 2*</td>
<td>30 ± 2*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>APO B, mg/dl</td>
<td>141 ± 5</td>
<td>133 ± 4</td>
<td>116 ± 7*</td>
<td>0.008</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>0.4 (0.2–0.8)</td>
<td>1.4 (0.8–2.4)*</td>
<td>9.2 (4.6–18.3)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>0.52 (0.24–1.09)</td>
<td>1.8 (1.1–2.98)*</td>
<td>4.99 (2.64–9.43)*</td>
<td>0.0007</td>
</tr>
<tr>
<td>Adiponectin, μg/ml</td>
<td>11.8 (7.2–19.4)</td>
<td>9.3 (6.5–13.3)</td>
<td>8.5 (5.4–13.3)</td>
<td>0.59</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>4.5 (2.4–8.6)</td>
<td>6.4 (3.9–5.9)</td>
<td>3.3 (1.9–5.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>6.8 (4.8–9.6)</td>
<td>6.4 (5.0–8.3)</td>
<td>8.2 (5.9–11.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>5.7 ± 1.1</td>
<td>6.2 ± 0.8</td>
<td>8.3 ± 1.0</td>
<td>0.35</td>
</tr>
<tr>
<td>PAI-1, mg/ml</td>
<td>295 ± 43</td>
<td>296 ± 30</td>
<td>205 ± 38</td>
<td>0.77</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>24.8 ± 3.5</td>
<td>25.8 ± 2.5</td>
<td>23.4 ± 3.1</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Values are means ± SE or geometric mean and confidence interval; n, no. of subjects; LDL, low density lipoprotein; HDL, high density lipoprotein; chl, cholesterol; Apo, apolipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1. ANOVA and post hoc Tukey-Kramer test: P < 0.05 vs. control (*) and stable CAD (†).
TaqMan gene expression assay on Demand (Applied Biosystems) on the Fast 7900HT Real-Time PCR. A preamplification uniformity test was performed, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene.

Relative quantitation values were expressed using the $2^{-\Delta\Delta C_{t}}$ (where $C_{t}$ is cycle threshold) method as fold changes in the target gene normalized to GAPDH and related to the average expression of the control group. The PCR efficiency in all runs was close to 100%, and all samples were tested at least in duplicate.

Immunoassays for serum and cultured medium adipokines. Concentrations of cytokines and chemokines in CM were assayed with Luminex xMAP technology. Adiponectin, resistin, total PAI-1, IL-6, leptin, TNF-α, and MCP-1 levels were measured both in total serum and culture medium simultaneously by Human Adipokine kits (Linco Research, St. Charles, MO) with a Bioplex (Bio-Rad, Milan, Italy), except for serum IL-6 that was assayed by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine HS: R&D Systems, Minneapolis, MN). The concentration of IL-10 in CM was assayed by commercially available ELISA kits (Quantikine HS; R&D Systems). Assays were performed according to the manufacturer’s protocol. Intra-assay variability was <12%, whereas interassay variability was <15%.

Endothelial cell isolation and culture. HUVEC were isolated from human cord by collagenase treatment as described (7) and cultured in 1% gelatin-coated flasks (Falcon; Becton-Dickinson, Bedford, MA) using endotoxin-free Medium 199 (BioWhitaker, Cambrex Bio Science Verviers, Belgium) containing 20% heat-inactivated FBS (HyClone, Logan, UT), 1% bovine retinal-derived growth factor, 90 μg/ml heparin, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Biochrom, Berlin, Germany) (complete medium, TCM). All experiments were carried out with HUVECs at passage 1–4.

In vitro permeability assay. The assay was carried out using Transwell cell culture chambers (0.4 μm polycarbonate filters), as described (8). Briefly, HUVECs were grown to reach the confluence in the upper compartment and then exposed for 24 h to either CM or recombinant resistin diluted in TCM, in the presence or absence of rabbit anti-resistin serum (1:200 dilution) (Phoenix Pharmaceuticals, Burlingame, CA). TNF-α (5 ng/ml) diluted in TCM was used as a positive control. To avoid transcellular transport and saturate albumin receptors, HUVECs were exposed for 5 min to Medium 199 containing 1.5 mg/ml human serum albumin (Farma-Biagini, Lucca, Italy). After that, saline (0.9% NaCl) was added to the lower chamber and 1% BSA to the upper photometer, in a Bio-Rad microplate reader.

Histology and confocal microscopy. Two opposite faces of frozen EAT blocks were sectioned and submitted to hematoxylin/eosin (H&E) and confocal microscopy. Morphology was determined on H&E by a pathologist working blindly under an Eclipse55i microscope equipped with a DS-L1 camera (Nikon, Tokyo, Japan). For confocal microscopy, EAT sections fixed in acetone (10 min, RT) and permeabilized with 0.05% Triton X-100 in PBS (10 min, RT) were incubated in Mouse-anti-Human CD68 (DAKO Cytomation, Carpinteria, CA) (diluted 1:100, overnight, 4°C), revealed either with Alexa Fluor 488-Rabbit anti-Mouse IgG (single staining) or AlexaFluor 594-Goat-anti-Mouse IgG (double staining), diluted 1:500 for 45 min at RT (Molecular Probes, Eugene, OR). Doubly stained sections were submitted to Rabbit-anti-resistin (diluted 1:100, 2h, RT) revealed by AlexaFluor 488-Goat-anti-rabbit IgG. Nuclei were stained with DAPI (0.2 μM, 10 min, 37°C). The slides were examined under a Leica TCS SP2 AOBS confocal microscope (Leica Microsystems, Wetzlar, Germany). CD68+ cells were counted on 6–10 two-dimensional Free Projection Max obtained from Z-series, and the percentage of CD68+/trabecular cells was calculated.

Statistical analysis. Continuous variables are presented as means ± SE or geometric mean and confidence interval. Variables with skewed distribution were log transformed. Statistical comparisons among the three groups were performed using ANOVA followed by a two-tailed post hoc Tukey-Kramer test. Associations between variables were determined with the Pearson or Spearman correlation coefficients for continuous measurements. Expression differences were considered significant at $P < 0.005$, according to Bonferroni adjustment for multiple comparisons. All analyses were performed using the JMP6 statistical package (SAS Institute, Cary, NC).

RESULTS

General characteristics. The general characteristics of the patients in the three groups are summarized in Table 1. The two groups of patients who underwent CABG (i.e., stable CAD and ACS) were matched for age and BMI. No differences between stable CAD and ACS groups were found for either established risk factors (dyslipidemia, diabetes, hypertension, current smoking, and family history of CAD) or extent of disease, whereas a higher consumption of aspirin, nitroglycerin, heparin, and insulin was seen in ACS. Subjects in the control group were younger and had less risk factors and consumption of medications than stable CAD and ACS groups.
No differences were observed for glucose and lipid variables between stable CAD and ACS patients, with the exception of lower high density lipoprotein cholesterol and apolipoprotein A-I in ACS (Table 2). Low density lipoprotein cholesterol was lower in both stable CAD and ACS than in controls. Serum concentrations of hsCRP and IL-6 were also significantly different among the three groups, being lower in controls and higher in ACS patients. Most ACS patients were TnI negative (<0.2 ng/ml), whereas low (0.2–0.4 ng/ml) and high (>1.0 ng/ml) serum TnI levels were observed in 28 and 16% of ACS patients, respectively. No significant differences in serum concentrations of all measured variables (adiponectin, leptin, resistin, TNF-α, MCP-1, and PAI-1) were observed in the three groups.

Gene expression of EAT adipokines. To investigate possible differential expression between stable CAD and ACS patients, mRNA expression levels of EAT adipokines were evaluated. Values are given as fold increase relative to the control group, and differences between groups were considered significant at \( P < 0.005 \) (Bonferroni’s correction for multiple comparison) (Fig. 1). Resistin was significantly overexpressed (3.0-fold) in ACS but not in stable CAD patients \( (P = 0.003) \) (Fig. 1A). This finding was completely independent of TnI \( (P = 0.003) \) and only partially influenced by either CRP or IL-6 \( (P = 0.02 \) for both) levels. IL-6 was overexpressed in both ACS and stable CAD (5.1- and 3.3-fold, respectively). PAI-1 and MCP-1 were also highly expressed (2.1-fold) in ACS patients but without any significant difference from the stable CAD group, which showed an increase of \(<2.0\)-fold.

Significant changes were also observed in the expression of CD68 and IL-10 in ACS compared with both controls and stable CAD and in that of adiponectin, leptin, and MIF in both ACS and stable CAD compared with controls (Fig. 1B). However, these changes are negligible (between 0.5- and 2.0-fold) and, therefore, of likely questionable biological significance.

Light and confocal microscopy of EAT. Figure 2 shows microscopy of EAT samples from the three groups of patients. The intact morphology of adipose tissue was shown by H&E (Fig. 2A). Confocal microscopy demonstrated the presence of CD68\(^+\) cells along the septa (Fig. 2B). Quantitative analysis

A

Control

Stable CAD

ACS

B

CD68 & DAPI

CD68 & DAPI

CD68 & DAPI

C

% CD68\(^+\) cells

Control (n=6)  Stable CAD (n=7)  ACS (n=10)

Fig. 2. Light and confocal microscopy of EAT specimens. A: microscopic features of hematoxylin/eosin (H&E)-stained EAT, displaying a regular network of septa, and portions of the pericardial sac. B: confocal microscopy of macrophage (CD68\(^+\)) distribution in EAT. A higher percentage of macrophages is present in ACS than in both stable CAD and control groups, and the cells appear randomly distributed among the septa. Scale bars indicate the magnifications. C: histogram (mean ± SE) of macrophage (CD68\(^+\)) quantification in the three groups. \( P < 0.05 \) vs. control (*) and stable CAD ($).
showed a significantly higher percentage of CD68² macrophages per trabecular cells in ACS compared with the other groups (27.4 ± 2.5 vs. 16.7 ± 1.5 stable CAD, and 17.3 ± 3.6 for the control group; \( P = 0.01 \) by ANOVA; \( P < 0.05 \) vs. both stable CAD and control groups by post hoc Tukey-Kramer test) (Fig. 2C). Moreover, we observed that the distribution of resistin on EAT mostly colocalized with CD68² cells, and the signal is higher in ACS (Fig. 3). However, a slight signal could also be detected in the interstitial space and in other cell types.

**Protein secretion of EAT adipokines.** We then investigated the release of adipokines by EAT in the three groups of patients by culturing EAT fragments for 24 h and then assessing adipokine concentration by Luminex technology. The concentration of resistin in 24-h CM was significantly higher in ACS compared with control and stable CAD groups (Table 3). A slight signal could also be detected in the interstitial space and in other cell types.

### Table 3. Inflammatory markers and adipokines in 24 h cultured medium

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stable CAD</th>
<th>ACS</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>59 (36–97)</td>
<td>81 (57–113)</td>
<td>122 (76–194)</td>
<td>0.22</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.23 (0.09–0.65)</td>
<td>0.39 (0.20–0.75)</td>
<td>0.54 (0.22–1.29)</td>
<td>0.54</td>
</tr>
<tr>
<td>Resistin</td>
<td>0.05 (0.02–0.15)</td>
<td>0.16 (0.08–0.32)*</td>
<td>0.74 (0.28–1.89)*†</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.24 (0.10–0.53)</td>
<td>0.73 (0.41–1.28)*</td>
<td>1.44 (0.65–3.16)*†</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.2 (1.8–5.9)</td>
<td>2.7 (1.9–3.8)</td>
<td>6.0 (3.8–9.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.99 (0.43–2.28)</td>
<td>1.38 (0.77–2.50)</td>
<td>1.45 (0.67–3.16)</td>
<td>0.72</td>
</tr>
<tr>
<td>PAI-1</td>
<td>0.70 (0.25–1.96)</td>
<td>1.31 (0.65–2.64)</td>
<td>1.81 (0.74–4.47)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Values are geometric mean and confidence interval; \( n \), no. of subjects. Units are ng·ml⁻¹·100 mg tissue⁻¹, except for IL-10 (pg·ml⁻¹·100 mg tissue⁻¹). ANOVA and post hoc Tukey-Kramer test: \( P < 0.05 \) vs. control (*) and stable CAD (†).
than stable CAD patients, whereas IL-6 concentration was higher in both CABG groups compared with controls, without any significant difference between ACS and stable CAD (Table 3). No differences among the three groups were observed in the concentration of adiponectin, leptin, IL-10, MCP-1, and PAI-1 in 24-h culture medium. TNF-α concentrations in the medium were below the sensitivity of the method (0.14 pg/ml).

**HUVeC permeability after incubation with supernatant of cultured EAT.** Considering the well-known direct proinflammatory effect of adipokines on vascular endothelial cells, we investigated whether CM could affect endothelial function. For this purpose, we selected the in vitro model of endothelial permeability. The integrity of the HUVeC monolayer was altered after a 24-h exposure to CM-ACS, as shown by significantly increased paraendothelial transit of albumin compared with CM-stable CAD and the CM control group (Fig. 4A). Of note, the average value of albumin transit obtained with CM-ACS was indistinguishable from that with TNF-α, a prototypic cytokine able to affect endothelial permeability.

Endothelial cell permeability was significantly correlated to resistin \((r = 0.57, P = 0.007)\) and IL-6 \((r = 0.39, P = 0.05)\) concentration in the culture medium. Partial correlation coefficient adjusted by concentration of all adipokines remained significant only between endothelial cell permeability and resistin \((r = 0.56; P < 0.005)\).

To test for the relative contribution of resistin to the functional impairment of the monolayer, a resistin blocking antibody was added to CM-ACS, in a concentration that actively resulted in a titration curve with the recombinant resistin protein (not shown) and that specifically abolished resistin-induced (Fig. 4B), but not TNF-α-induced (Fig. 4C), endothelial permeability. Notably, anti-resistin serum completely abrogated the increased endothelial cell permeability induced by CM from ACS patients (Fig. 4B). The addition of anti-resistin serum alone did not perturb the integrity of the monolayer (Fig. 4C). These findings support the view that resistin is a major inducer of endothelial damage, among the other adipokines released from EAT.

**Discussion**

The present study is focused on local events and demonstrates that EAT of patients with ACS is characterized by a specific increased production of resistin, occurring in an inflammatory milieu also found in patients with stable CAD and mostly represented by elevated IL-6. Here we also show that the elevated concentration of resistin generated in EAT of ACS patients seems to be a major determinant of increased endothelial cell permeability in vitro, indicating a potential pathogenetic mechanism linking EAT to potential culprit lesions in coronary arteries of ACS patients.

**Resistin gene expression and concentration in EAT of ACS.** Our data indicate that EAT of ACS patients had a higher expression and secretion of resistin and an increased presence of CD68⁺ cells compared with either patients with stable CAD or subjects without CAD. Confocal analysis revealed that CD68⁺ cells, whose presence was independent of age and adiposity, colocalized with resistin, thus suggesting that these cells are responsible for resistin production.

Resistin belongs to a family of cysteine-rich secretory proteins called FIZZ/RELM (found in inflammatory zone/resistin-like molecules). The exact role of resistin is currently unclear. In rodent, resistin is derived almost exclusively from adipocytes, whereas macrophages are the major source of resistin in humans (6). Plasma resistin levels have been found to correlate with markers of inflammation as well as coronary atherosclerosis (24) and abdominal aortic aneurysm (10) and to be increased in incident heart failure (9) and ACS (19). Plasma resistin levels have also been recently shown as a predictor of mortality in patients with acute myocardial infarction (17).
However, greater concentrations of resistin have been associated with coronary heart disease CAD in some (1, 22–24) but not in other studies, including ours (18, 29).

The present study also confirms the role of a constant local EAT inflammation in the whole heterogeneous group of high-risk CAD patients, since additional adipokines are differentially expressed compared with subjects without CAD. Previous studies have shown lower levels of adiponectin (5, 14) and higher levels of TNF-α, IL-6, leptin, and visfatin in EAT of patients with CAD compared with controls without CAD (5). Besides these adipokines, we also demonstrate a slight increased expression of PAI-1 and MCP-1 in ACS patients, though not significantly different from stable CAD patients. It has been proposed that this proinflammatory adipokine profile in EAT of CAD patients could lead to amplification of vascular inflammation in atherosclerotic coronaries or be proinflammatory, per se (12).

**Resistin-induced in vitro endothelial cell permeability in ACS.** Our study also demonstrates that high concentrations of resistin generated in CM from EAT of ACS patients profoundly influence in vitro endothelial function by significantly increasing endothelial cell permeability. Recently, many reports have addressed the contribution of periorgans fat to the development of CAD, mediated via adipokine action on the vascular endothelium (25). Several authors have shown detrimental effects of resistin on vascular endothelial cells, supporting a role of resistin in atherosclerosis. Resistin has been shown to be secreted by monocytes/macrophages infiltrating the arterial wall (15) and to induce endothelial dysfunction by upregulating vascular cell adhesion molecule-1 and endothelin-1 (28). In addition, resistin also appears to induce human endothelial cell proliferation and migration in vitro, thus promoting capillary-like tube formation in angiogenesis-associated vascular disorders (21) and to have a proapoptotic effect on endothelial progenitor cells but not on mature human aortic endothelial cells, even at a high dose (31). More recently, it has also been shown that adipocyte-secreted factors induce secretion of various proinflammatory and chemotactic cytokines from endothelial cells (27). Our data strongly support the findings described by Sommer et al. (27), and add the notion that resistin is indeed an important adipokine that might directly and specifically impair endothelial permeability. Thus our data reveal an additional mechanism of endothelial dysfunction promoted by resistin. Indeed, supernatants derived from patients with ACS and containing the highest concentrations of resistin significantly affected the barrier function of HUVEC. This effect was abrogated by the addition of neutralizing anti-resistin monoclonal antibody.

We therefore hypothesize that EAT-generated resistin, which could reach the endothelium of coronary arteries via paracrine or vasocrine signaling, might have a role in sustaining hyperpermeability in vivo and therefore play an important role in the development of ACS.

We conclude that EAT of patients with ACS is characterized by an increased production of resistin compared with either patients with stable CAD or individuals with angiographically normal coronary arteries. The increased production of this adipokine in EAT of patients with ACS occurs in a milieu of chronic local inflammation that present in both stable and unstable CAD patients. The specific mechanisms driving the increased production of resistin in EAT of ACS patients remain to be elucidated. Moreover, the question whether simple overabundance or abnormal activation of macrophages is responsible of their detrimental metabolic action is still open. Strategies targeting specific macrophage recruitment and activation or resistin itself could be promising options for the treatment of ACS.

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**DISCLOSURES**

None.

**REFERENCES**


