Human Epicardial Adipose Tissue Is a Source of Inflammatory Mediators

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Background—Inflammatory mediators that originate in vascular and extravascular tissues promote coronary lesion formation. Adipose tissue may function as an endocrine organ that contributes to an inflammatory burden in patients at risk of cardiovascular complications. In this study, we sought to compare expression of inflammatory mediators in epicardial and subcutaneous adipose stores in patients with critical CAD.

Methods and Results—Paired samples of epicardial and subcutaneous adipose tissues were harvested at the outset of elective CABG surgery (n=42; age 65±10 years). Local expression of chemokine (monocyte chemotactic protein [MCP]-1) and inflammatory cytokines (interleukin [IL]-1β, IL-6, and tumor necrosis factor [TNF]-α) was analyzed by TaqMan real-time reverse transcription–polymerase chain reaction (mRNA) and by ELISA (protein release over 3 hours). Significantly higher levels of IL-1β, IL-6, MCP-1, and TNF-α mRNA and protein were observed in epicardial adipose stores. Proinflammatory properties of epicardial adipose tissue were noted irrespective of clinical variables (diabetes, body mass index, and chronic use of statins or ACE inhibitors/angiotensin II receptor blockers) or plasma concentrations of circulating biomarkers. In a subset of samples (n=11), global gene expression was explored by DNA microarray hybridization and confirmed the presence of a broad inflammatory reaction in epicardial adipose tissue in patients with coronary artery disease. The above findings were paralleled by the presence of inflammatory cell infiltrates in epicardial adipose stores.

Conclusions—Epicardial adipose tissue is a source of several inflammatory mediators in high-risk cardiac patients. Plasma inflammatory biomarkers may not adequately reflect local tissue inflammation. Current therapies do not appear to eliminate local inflammatory signals in epicardial adipose tissue.

Key Words: inflammation ■ atherosclerosis ■ coronary disease

Large population-based studies, detailed characterization of human vascular lesions, and experimental laboratory investigations have all provided abundant evidence that inflammation plays a central role in the development and progression of atherosclerosis. Epidemiological data have linked circulating levels of inflammatory cytokines (eg, interleukin [IL]-6 and tumor necrosis factor [TNF]-α) or their hepatic product, C-reactive protein, with long-term cardiovascular risk in apparently healthy populations and in those with already established coronary artery disease (CAD). In addition to a vascular origin, inflammatory mediators may also originate from remote extravascular sources, thus providing a mechanistic explanation for increased cardiovascular risk in certain patient populations, including not only patients with chronic infections or chronic inflammation (eg, rheumatoid arthritis) but also insulin-resistant individuals who exhibit increased release of cytokines from adipose tissue.

In the vasculature, inflammatory signals are usually linked to blood-borne cells (eg, macrophages and T cells) retained in the intima and activated resident cells of vascular origin. Interestingly, inflammatory mediators originating outside the coronary artery are also capable of inducing compositional changes in the inner layer of intima. The possibility of "outside-to-inside" cellular cross talk is further underscored by neutralizing effects of adventitial oxidative stress on endothelial nitric oxide. In this context, the presence of metabolically active adipose stores that surround epicardial coronary arteries could contribute to the inflammatory burden. To this end, the results of the present study demonstrated significantly higher expression of chemokine (monocyte che-
Tables and figures are not shown in this natural text representation.
change" and defined as ratio between normalized intensities of the 2 arrays derived from the same patient.

**Immunohistochemistry**

Frozen sections (10 μm) were air-dried for 15 minutes and immersed in xylene for 10 minutes to remove the fat. The sections were then hydrated in descending grades of alcohol and stained with hematox- ylin and eosin. Selected serial sections were subjected to immuno- histochemistry with the Universal Elite ABC kit (Vector Laborato- ries) according to the manufacturer’s protocol. Briefly, sections were incubated with 0.3% H2O2 in methanol for 30 minutes followed by blocking with 5% horse or goat serum. After they were washed in PBS, sections were incubated with primary antibodies for 1 hour in a moisture chamber. Afterward, the slides were incubated with secondary antibodies for 30 minutes followed by avidin-biotin for 30 minutes. Sections were then exposed to DAB and counterstained with hematoxylin. The following antibodies were used: CD3 (T cell, 1:50, Dako), and tryptase (mast cell, 1:50, Novocastra).

**Statistical Analysis**

Continuous variables are presented as mean±SE. Variables with skewed distribution were log transformed. To account for zero values, one tenth of the lowest nonzero value derived from each data set was added to measured values, which permitted log transformation of all patient samples and retained their rank ordering. Only complete sets (ie, pairs) of epicardial and subcutaneous adipose tissue results are reported. Statistical comparisons and confidence interval estimates of expression levels for epicardial and subcutaneous adipose samples were performed with the paired Wilcoxon test. Associations between epicardial fat expression levels and plasma markers or clinical variables were determined with the Pearson and Spearman correlation coefficients for continuous measurements and 2-sample Wilcoxon tests for binary categorical data. Expression differences and associations were considered significant at P<0.05. For multiple comparisons, Bonferroni adjustment was used. For the microarray substudy, mean values for the probe sets that were located at 3’ end are reported. Ratios producing unidirectional change at P<0.05 in at least 8 of 11 total paired samples were considered as differentially expressed genes.

**Results**

**Patient Characteristics**

Table 1 describes demographic and clinical characteristics of the study group. As expected, the majority of patients undergoing CABG surgery were elderly, were male (86%), and had multivessel CAD with a high prevalence of obesity (50%), dyslipidemia (55%), hypertension (69%), and diabetes (57%). Table 2 summarizes laboratory values for serum cholesterol, glucose, insulin, and plasma inflammatory biomarkers.

**Inflammatory Burden in Epicardial Adipose Tissue**

As shown in Figure 1, expression data revealed significantly higher levels of all inflammatory mediators in epicardial versus subcutaneous adipose tissue (mRNA: IL-1β P<0.02, IL-6 P<0.001, MCP-1 P<0.002, and TNF-α P<0.001; protein: IL-1β P<0.001, IL-6 P<0.001, IL-6sR P<0.001, MCP-1 P<0.001, and TNF-αP<0.001). Table 3 summarizes the fold increase (median and 95% CI) of inflammatory biomarkers in epicardial adipose stores, illustrating regional proinflammatory properties of epicardial adipose tissue. When a priori selected categorical variables (ie, gender, smoking, hypertension, diabetes, history of acute coronary syndrome, extent of CAD, ejection fraction <30%, statin or ACE inhibitor/angiotensin II receptor blocker therapy) and continuous variables (ie, age and body mass index) were analyzed, no significant associations with epicardial adipose tissue inflammation were detected. Likewise, homeostasis model assessment–insulin resistance (for nondiabetics) was not predictive of local inflammatory burden in epicardial fat. Overall, inflammatory mediators in epicardial adipose tissue showed variable correlations with their respective levels in subcutaneous adipose stores, with the strongest correlation observed for MCP-1 (Spearman r=0.60, P<0.001).

Next, we examined whether plasma concentrations of inflammatory biomarkers (IL-6, IL-6sR, MCP-1, and TNF-α) correlate with epicardial tissue inflammation. Circulating inflammatory biomarkers showed no significant correlations with their epicardial adipose tissue concentrations, except for a weak correlation between plasma and epicardial IL-6sR protein levels (Spearman r=0.45, P<0.01) that became significant after adjustment for multiple comparisons. Likewise, serum HDL levels demonstrated a weak inverse correlation with epicardial expression of IL-6sR (Spearman r=−0.47, P<0.005), which became insignificant after adjustment for multiple comparisons. There were no detectable associations between serum LDL levels and epicardial inflammation.

To broaden characterization of different fat depots, we performed microarray analysis in a subset of paired samples (n=11). A total of 1003 genes were differentially expressed between epicardial and subcutaneous adipose tissues (P<0.05, unidirectional change in at least 8 of 11 patients). Among differentially expressed genes, 805 genes were up-regulated and 198 were downregulated in epicardial adipose tissues. Genes were annotated and grouped on the basis of their functions using PubMed. As shown in Figure 2, cluster analysis indicated that differentially expressed genes could be classified into various subgroups on the basis of the similarity of their expression. In epicardial adipose stores, the upregulated genes included inflammatory (eg, chemokine ligand 21) and immune response (eg, immunoglobulin-κ 1 to 39) genes, whereas downregulated genes consisted of adipocyte-related genes (eg, adiponectin 2). Table 4 shows selected

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**TABLE 2. Laboratory Measurements**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>148.1 ± 7.5</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>31.5 ± 1.6</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>91.9 ± 6.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>167.3 ± 27.7</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>130.9 ± 6.0</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Plasma inflammatory markers, pg/mL</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>6.01 ± 0.86</td>
</tr>
<tr>
<td>IL-6sR</td>
<td>21.54 ± 1.29</td>
</tr>
<tr>
<td>MCP-1</td>
<td>110.38 ± 29.65</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.99 ± 0.71</td>
</tr>
</tbody>
</table>

HOMA-IR indicates homeostasis model assessment–insulin resistance.
inflammation-related genes that were upregulated in epicardial adipose tissue.

Retention of Inflammatory Cells in Epicardial Adipose Tissue

Differential expression of chemokines (Figure 1 and Table 4) in epicardial adipose tissues could result in increased retention of inflammatory cells. To this end, epicardial and subcutaneous samples were examined by hematoxylin and eosin staining and immunohistochemistry (n=11). Compared with subcutaneous fat, epicardial fat showed thickened connective tissue septa with dense inflammatory cell infiltrates that extended to the perisepal areas of the fat lobules (Figure 3). Microvessels of epicardial fat contained variable degrees of leukocyte accumulation, whereas no sign of cellular retention was observed in subcutaneous fat. When specific inflammatory cell markers were used, epicardial adipose tissue demonstrated presence of T lymphocytes (CD3+), macrophages (CD68+), and mast cells (tryptase+; Figure 4).

Discussion

The major findings of this study were as follows: (1) In patients with significant CAD, epicardial adipose tissue exhibited significantly higher levels of chemokine (MCP-1) and several inflammatory cytokines (IL-1β, IL-6, IL-6sR, and TNF-α) than subcutaneous fat. (2) Local inflammatory burden may not correlate with plasma concentrations of circulating cytokines. (3) Epicardial adipose tissue inflammation was independent of several clinical variables (obesity, diabetes, or chronic therapy with statins or ACE inhibitors).
Proinflammatory Properties of Adipose Tissue

Adipose tissue is a complex organ involved in the production of bioactive molecules. Studies to date have focused on obesity, in which higher expression of TNF-α or IL-6 in fat tissue correlates with insulin resistance. Adipocyte-derived TNF-α acts mainly in an autocrine fashion, impairing signaling via the insulin receptor and increasing lipolysis with the subsequent release of nonesterified fatty acids that contribute to insulin resistance in the peripheral tissues. In contrast, IL-6 accentuates systemic low-grade inflammation and hepatic production of C-reactive protein and inhibits lipoprotein lipase. Adipose tissue itself can be a source of C-reactive protein synthesis that inversely correlates with adiponectin, an endogenous adipocyte-derived anti-inflammatory protein. Although regional differences between different adipose stores (e.g., omental versus subcutaneous) have been reported in regard to inflammatory mediator expression (e.g., IL-6), comprehensive characterization of epicardial adipose tissue has not been undertaken. To the best of our knowledge, the present study is first to demonstrate inflammatory properties of epicardial adipose stores in patients with CAD independent of obesity or diabetes.

“Outside-to-Inside” Signaling and Putative Mechanisms

The signaling that originates from the vessel lumen or at the endothelial/intimal interface plays a key role in development of atherosclerotic lesions. There is growing evidence, however, that the changes in the adventitia or even perivascular tissues could also alter vascular homeostasis. The presence of inflammatory mediators in the tissues surrounding epicardial coronary arteries could lead to amplification of vascular inflammation, plaque instability via apoptosis (TNF-α), and neovascularization (MCP-1). In fact, periadventitial application of endotoxin, MCP-1, IL-1β, or oxidized LDL induces inflammatory cell influx into the arterial wall, coronary vasospasm, or intimal lesions, which suggests that bioactive molecules from the pericoronary tissues may alter arterial homeostasis. Other potential consequences of inflammatory reaction derived from epicardial adipose tissue could be beneficial, such as stimulation of an angiogenic response and development of collateral circulation in patients with obstructive CAD.

Several putative mechanisms could be put forward in regard to the observed characteristics of epicardial adipose tissue. First, impaired preadipocyte differentiation owing to conventional risk factors ought to be considered, although no

![Figure 2. Differential gene expression by cluster analysis: 1003 genes were grouped into clusters according to their expression pattern with TreeView software. Clustered gene expression data were displayed in color-coded grid, with individual paired samples ordered along x-axis and genes ordered along y-axis. Individual blocks in color-coded grid indicate fold change in expression of specific gene in epicardial and subcutaneous adipose samples from individual patients. Top, Colorimetric scale is shown: red indicates upregulation; green, downregulation; and black, no change. Dendrograms along x-axis represent hierarchical clustering of 11 paired samples based on similarity in gene expression. Examples of downregulated (adipose specific 2) or upregulated (T-cell differentiation protein, chemokine ligand 21, and immunoglobulin-κ 1 to 39) genes are shown from regions indicated by arrows.](http://circ.ahajournals.org/content/images/figure2_large.jpg)
apparent associations with clinical or laboratory variables were found. Second, regional ischemia and depressed myocardial function, with the subsequent increase in the redox state and cytokine expression, could activate oxidant-sensitive inflammatory signals in adjacent adipose stores.\textsuperscript{20,21} Third, preadipocyte differentiation to macrophages has been documented previously, although immunohistochemical characterization of epicardial fat suggests the presence of intravascular and infiltrating inflammatory cells of diverse origin (macrophages, mast cells, and T cells).\textsuperscript{22} They likely also included B cells, as suggested by high levels of immune...
noglobulin expression by microarrays (data not shown). Additional investigations of individual cellular components of epicardial tissue could provide information regarding their relative contribution to the inflammatory reaction. Fourth, the presence of inflammatory cells in adipose tissue could merely reflect the response to plaque rupture, analogous to inflammatory infiltrates in the adventitia and perivascular region adjacent to advanced atherosclerotic lesions.\(^2\)\(^3\)\(^4\) It is noteworthy, however, that epicardial tissue biopsy samples were obtained from randomly selected sites not necessarily in the vicinity of coronary lesions.

**Study Limitations**

Because the findings of this study are limited to patients with advanced CAD, the temporal relationship between inflammatory changes in epicardial adipose stores and the progression of CAD remains unknown. Ethical concerns prevented us from obtaining epicardial adipose tissue biopsy samples in patients without CAD undergoing heart surgery for other indications. It is also noteworthy that epicardial biopsy samples from patients undergoing heart transplantation for nonischemic cardiomyopathy are not appropriate for comparisons because of high myocardial expression of inflammatory genes.\(^2\)\(^3\)\(^4\) The absence of significant correlations between clinical risk factors or circulating levels of inflammatory biomarkers and enhanced inflammation within the epicardial adipose tissue should be interpreted with caution. The sample size was modest for association studies and may have mitigated against demonstrating statistical associations.

**Conclusions**

This study demonstrated augmented inflammatory responses in epicardial adipose tissue in patients with significant CAD. This response was independent of body mass index or diabetes. Importantly, inflammatory signals from epicardial adipose tissue were neither strongly correlated with plasma inflammatory biomarkers nor attenuated by chronic treatment with conventional cardiovascular therapies, including statins or ACE inhibitors/angiotensin II receptor blockers.

**Acknowledgments**

This study was supported by grants from the National Institutes of Health (HL-60672), American Diabetes Association, and John S. Sharpe Foundation. The authors thank Mark E. Burgert and Kay S. Tatsuoka from GlaxoSmithKline for assistance with statistical analysis.

**References**

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Circulation. 2003;108:2460-2466; originally published online October 27, 2003;
doi: 10.1161/01.CIR.0000099542.57313.C5
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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