Brief Report

Attenuated adiposopathy in perivascular adipose tissue compared with subcutaneous human adipose tissue

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Abstract

BACKGROUND: We hypothesized that human perivascular and subcutaneous adipose tissues hold distinct phenotypic signatures. We also evaluated the impact of clinical parameters on the adipose phenotype. Our overall goal is to understand the determinants of adipose biology so that this tissue can be manipulated therapeutically to lessen peripheral vascular disease.

METHODS: Perivascular and subcutaneous adipose tissues were collected from patients undergoing lower-extremity amputation (n = 27) and protein assayed for proinflammatory mediators (ie, interleukin 6, interleukin 8, leptin, tumor necrosis factor α, monocyte chemoattractant protein-1, and resistin), atheroprotective adiponectin, and the fibrinolysis inhibitor plasminogen activator inhibitor-1.

RESULTS: Leptin (2.7-fold, P = .015), TNF-α (2.2-fold, P = .013), MCP-1 (1.5-fold, P = .047), and adiponectin (1.8-fold, P = .004) were more abundant in subcutaneous vs perivascular adipose tissue. Age positively correlated with perivascular adipose tissue PAI-1 expression (β = .64, P = .042), and hyperlipidemia negatively correlated with perivascular adiponectin (β = −1.18, P = .039).

CONCLUSIONS: Human perivascular and subcutaneous adipose tissues hold distinct phenotypic signatures. In amputation patients, the subcutaneous adipose tissue proinflammatory phenotype was relatively attenuated in perivascular adipose tissue.

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Although historically viewed as a relatively inert tissue limited to roles in thermoregulation, musculoskeletal protection, and energy storage, adipose tissue is increasingly recognized as a highly active tissue with endocrine and immune functions.1,2 Adipose localizes focally throughout the body, and it can be grouped into the following distinct gross anatomic depots: visceral, subcutaneous, and perivascular.3,4,5,6 Although visceral adipose tissue with its relationship to “central obesity” has been implicated as a highly metabolically active compartment, perivascular adipose tissue also has become increasingly important under the “outside-in” hypotheses of vascular disease.4,6 Surrounding systemic vessels with the exception of the cerebral circulation, perivascular adipose tissue exhibits emerging links to
vascular pathologies.\textsuperscript{3,4,6,7,8} To date, few direct interroga-
tions of human perivascular adipose tissue have been com-
pleted,\textsuperscript{9,10} and often adipose knowledge is founded on
animal models or serum biomarkers coupled with imaging.

Our overall goal is to understand the determinants of
adipose biology so that this tissue can be manipulated thera-
petically to lessen vascular disease. In view of their theorized
biologic roles, we hypothesized that perivascular and subcu-
taneous adipose tissue hold distinct phenotypic signatures. To
test this hypothesis, appropriate institutional review board
approvals were obtained for live tissue harvest in the operating
room from patients undergoing below- and above-knee lower-
extremity amputations performed for unreconstructable
chronic (all more than 1 month) critical limb ischemia or an
unsalvageable foot, and we assayed key biologic mediators
representing inflammatory signaling networks. Immediately
after amputation, fresh adipose was collected by surgeons
portions of the specimens at a level that was judged as likely to
heal by the attending surgeon. A portion of these patients were
also examined in a separate report.\textsuperscript{11}

Protein extraction was performed with a detergent-free
solution. Briefly, adipose was disrupted and homogenized in
ice-cold phosphate-buffered saline with protease inhibitors.
The homogenate was centrifuged (2,000 \( g \), 5 minutes at room
temperature), the top fat layer was discarded, and then the
supernatant was centrifuged again (10,000 \( g \), 10 minutes at
4°C). The supernatants containing the soluble proteins
were finally collected for subsequent assays. The total pro-
tein concentration was determined via a Bradford protein
assay.

Selected adipokines and adipose-derived hormones
(i.e., interleukin [IL]-6, IL-8, leptin, tumor necrosis factor-\( \alpha \)
[TNF-\( \alpha \)], monocyte chemoattractant protein-1 (MCP-1), adi-
ponectin, resistin, and plasminogen activator inhibitor-1
(NAI-1)) were quantified using a microsphere bead assay
(Luminex Corporation, Austin, TX), and concentrations were
normalized to the total protein. IL-6, IL-8, leptin, TNF-\( \alpha \),
MCP-1, resistin, and PAI-1 are generally associated with a
proinflammatory state. Adiponectin is a solely adipose-derived
hormone that protects against diabetes, obesity, and atheroscle-
rosis. Distinct corresponding tissue sections were processed via
formalin fixation and paraffin embedding, and histologic anal-
ysis was performed.

Univariate comparisons for equality of means were
performed with paired \( t \) tests to compare adipocyte-related
mediator expression between the 2 adipose depots in each
patient. Beyond comparisons between the subcutaneous and
perivascular samples, we also evaluated the impact of
standard clinical parameters on the adipose phenotype. Mul-
tivariate linear regression analysis was used to associate
clinical factors with mediator levels.

As is typical of major amputation patients, the results
showed that this sample represented a medically ill patient
population (\( N = 27 \), Table 1). Histologically, there were
minimal differences between the perivascular and subcuta-
neous adipose tissue samples; both showed scattered fat ne-
crosis and minimal chronic inflammation (Fig. 1A and B).
In comparison with subcutaneous adipose tissue, perivascu-
lar adipose tissue displayed a generally less inflamed phe-
notype, with statistically significant less leptin, TNF-\( \alpha \),
MCP-1, and adiponectin (Fig. 1C). There were also trends
for less IL-6 and IL-8 in perivascular adipose tissue.

Multiple linear regression analysis did not identify any
clinical predictors of the phenotype for subcutaneous adi-
pose including body mass index. Age positively correlated
with perivascular PAI-1 expression (\( \beta = .64, P = .042 \)), and
a history of hyperlipidemia was negatively associated with
perivascular adipose adiponectin (\( \beta = -1.18, P = .039 \)).

Perivascular adipose tissue holds particular relevance to
vascular biology in view of its tissue mass, anatomic
proximity, and emerging signaling role in inflammation and
vascular pathologies.\textsuperscript{3,4,6,7} Our results defined specific
human phenotypic signatures in an amputation patient co-
hort, with the perivascular adipose tissue displaying rela-
tively less inflammation (less adiposopathy\textsuperscript{1}) in totality.

Adipokines and adipose-derived hormones are impli-
cated in obesity, diabetes, and cardiovascular disease.\textsuperscript{1,2}
The relative disparity between subcutaneous and perivascu-
lar adipose tissue expression of the adipose-derived hor-
mones and adipokines highlights the heterogeneity of

\begin{table}[h]
\centering
\caption{Cohort clinical characteristics (\( N = 27 \))}
\begin{tabular}{ll}
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\textbf{Age} & \textbf{Mean ± SEM or percentage (n)} \\
\hline
\textbf{Male sex} & 71.3 ± 2.9 y \\
\textbf{Ethnicity} & \textbf{70 (19)} \\
\textbf{White} & 56 (15) \\
\textbf{Black} & 26 (7) \\
\textbf{Hispanic} & 18 (5) \\
\hline
\textbf{BMI} & 24.6 ± 1.2 kg/m\(^2\) \\
\textbf{Diabetes} & 63 (17) \\
\textbf{Hypertension} & 93 (25) \\
\textbf{Hyperlipidemia} & 70 (19) \\
\textbf{Coronary artery disease} & 67 (18) \\
\textbf{Congestive heart failure} & 30 (8) \\
\textbf{Cerebrovascular disease} & 26 (7) \\
\textbf{Renal disease} & 48 (13) \\
\textbf{Pulmonary disease} & 37 (10) \\
\textbf{Smoking history} & 70 (19) \\
\textbf{Antithrombotic therapy} & 85 (23) \\
\textbf{Warfarin} & 26 (7) \\
\textbf{Calcium channel blocker} & 37 (10) \\
\textbf{\( \beta \)-blocker} & 89 (24) \\
\textbf{ACE inhibitor or ARB} & 41 (11) \\
\textbf{HMG-CoA reductase inhibitor} & 78 (21) \\
\textbf{Oral steroid} & 11 (3) \\
\hline
\end{tabular}
\end{table}

\textsuperscript{ACE = angiotensin-converting enzyme; ARB = angiotensin recep-
tor blocker; BMI = body mass index; HMG-CoA = 3-hydroxy-3-methyl-
glutaryl-CoA reductase inhibitor; SEM = standard error of the mean.}
body fat depots. Proximity may allow paracrine signaling mechanisms with the popliteal and tibial arteries from which the tissues were obtained, but it is unknown if the relatively less inflamed perivascular phenotype was a primary driver of a homeostatic response to the vascular disease in this cohort or in fact secondary to vascular disease.

Notably, clinical factors (including body mass index) did not predict the adipose phenotype with 2 exceptions: age in the case of PAI-1 and hyperlipidemia in the case of adiponectin in perivascular adipose tissue. Prior research has focused on imaging and serum levels of these mediators, which may not well reflect the quality of human adipose tissue itself.

Limitations are acknowledged. Certainly, comparisons with normal, surgically sampled human adipose tissue would add great power, but such tissues (especially perivascular) are not readily available. The sample size was relatively small, and some of the mediators exhibited high variability (eg, IL-8). We highlight relative protein levels but cannot make claims about biologic activity. The cellular origin of the assayed proteins is not delineated nor is the balance of synthesis/degradation, but the results do paint a general picture of the overall tissue phenotype. Our dataset does not have visceral adipose or serum for relative comparisons; however, these have been widely described in the literature. Finally, the findings in this medically ill cohort may not apply across all patient types, but this distinct subset presents a highly clinically relevant group.

In summary, although some biologic similarity was found between subcutaneous and perivascular adipose tissues, the differential exhibited within individual patients in adipokines and adipose-derived mediators supports the concept that these tissue compartments hold distinct biologic roles. Research strategies leveraging investigative opportunities in unfortunate clinical circumstances such as major amputation may move health care toward limb loss prevention and beyond.

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