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Attenuated Adiposopathy in Perivascular Adipose Tissue Compared to 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 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 pro-inflammatory mediators (IL-6, IL-8, leptin, TNF- α , MCP-1, resistin), athero-protective adiponectin, and the fibrinolysis inhibitor PAI-1.

Results—Leptin (2.7-fold, p=0.015), TNF- α (2.2-fold, p=0.013), MCP-1 (1.5-fold, p=0.047) and adiponectin (1.8-fold, p=0.004) were more abundant in subcutaneous versus perivascular adipose tissue. Age positively correlated with perivascular adipose tissue PAI-1 expression (β =0.64, p=0.042), and hyperlipidemia negatively correlated with perivascular adiponectin (β =−1.18, p=0.039).

Conclusions—Human perivascular and subcutaneous adipose tissues hold distinct phenotypic signatures. In amputation patients, the subcutaneous adipose tissue pro-inflammatory phenotype was relatively attenuated in perivascular adipose tissue.

Keywords

Adipose; adipokine; perivascular

While 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

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throughout the body, and it can be grouped into distinct gross anatomic depots: visceral, subcutaneous, and perivascular.³⁻⁶ While 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.^{3, 4, 6-8} To date few direct interrogations of human perivascular adipose tissue have been completed,^{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 therapeutically to lessen vascular disease. In view of their theorized biologic roles, we hypothesized that perivascular and subcutaneous adipose tissue hold distinct phenotypic signatures. To test this hypothesis, appropriate IRB 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 one 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 from both the subcutaneous and perivascular (peritibial or popliteal artery) locations, flash-frozen in liquid nitrogen, and stored at -80°C . To avoid the confounding influence of profound ischemia, we utilized adipose from the more proximal portions of the specimens, at a level which was judged as likely to heal by the attending surgeon.

Protein extraction was performed with a detergent-free solution. Briefly, adipose was disrupted and homogenized in ice-cold phosphate-buffer saline with protease inhibitors. The homogenate was centrifuged (2000g, 5 minutes at room temperature), the top fat layer discarded, and then supernatant was centrifuged again (10,000g, 10 minutes, at 4°C). The supernatants containing the soluble proteins were finally collected for subsequent assays. Total protein concentration was determined via a Bradford protein assay.

Selected adipokines and adipose-derived hormones (IL-6, IL-8, leptin, TNF- α , MCP-1, adiponectin, resistin and PAI-1) were quantified utilizing a micro-particle bead assay (Luminex Corporation, Austin, TX), and concentrations were normalized to total protein. IL-6, IL-8, leptin, TNF- α , MCP-1, resistin and PAI-1 are generally associated with a pro-inflammatory state. Adiponectin is a solely adipose-derived hormone that protects against diabetes, obesity, and atherosclerosis. Distinct corresponding tissue sections were processed via formalin fixation and paraffin embedding, and histological analysis was performed.

Univariate comparisons for equality of means were performed with paired *t*-tests to compare adipocyte-related mediator expression between the two adipose depots in each patient. Beyond comparisons between the subcutaneous and perivascular samples, we also evaluated the impact of standard clinical parameters on adipose phenotype. Multivariate 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 I). Histologically, there were minimal differences between the perivascular and subcutaneous adipose tissue samples; both showed scattered fat necrosis and minimal chronic inflammation (Figure 1A, B). In comparison with subcutaneous adipose tissue, perivascular adipose tissue displayed a generally less inflamed phenotype, with statistically less leptin, TNF- α , MCP-1, and adiponectin (Figure 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 adipose, including body mass index. Age positively correlated with perivascular PAI-1 expression ($\beta=0.64$, $p=0.042$), and a history of hyperlipidemia was negatively associated with perivascular adipose adiponectin ($\beta=-1.18$, $p=0.039$).

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.^{3, 4, 6, 7} Our results defined specific human phenotypic signatures in an amputation patient cohort, with the perivascular adipose tissue displaying relative less inflammation (less adiposopathy¹) in totality.

Adipokines and adipose-derived hormones are implicated in obesity, diabetes, and cardiovascular disease.^{1, 2} The relative disparity between subcutaneous and perivascular adipose tissue expression of the adipose-derived hormones and adipokines highlights the heterogeneity of 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 adipose phenotype with the 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 (e.g. 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, while 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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Figure 1.

Representative histology from Masson's trichrome stained sections of perivascular (A) and subcutaneous (B) adipose tissues. Scale bar = 200 μ m. Both show scattered fat necrosis and minimal chronic inflammation. (C) Paired comparisons of mediator protein levels between subcutaneous and perivascular adipose tissue compartments.

Table I

Cohort Clinical Characteristics (n=27)

	Mean ± SEM or Percentage (n)
Age	71.3±2.9 years
Male Sex	70% (19)
Ethnicity	
White	56% (15)
Black	26% (7)
Hispanic	18% (5)
BMI	24.6±1.2 kg/m ²
Diabetes	63% (17)
Hypertension	93% (25)
Hyperlipidemia	70% (19)
Coronary Artery Disease	67% (18)
Congestive Heart Failure	30% (8)
Cerebrovascular Disease	26% (7)
Renal Disease	48% (13)
Pulmonary Disease	37% (10)
Smoking History	70% (19)
Antiplatelet Therapy	85% (23)
Warfarin	26% (7)
Calcium Channel Blocker	37% (10)
Beta Blocker	89% (24)
ACE Inhibitor or ARB	41% (11)
HMG-CoA Reductase Inhibitor	78% (21)
Oral Steroid	11% (3)