Subcutaneous Adipose Tissue Cell Composition is Associated with Visceral Fat Volume

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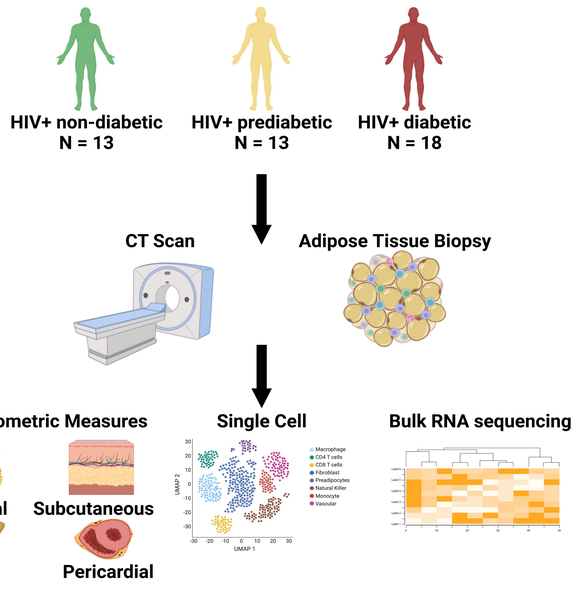
BACKGROUND

- Persons with HIV (PWH) have increased risk for ectopic deposition of lipids in multiple organs compared with HIV-negative persons
- This may in part, contribute to high risk for cardiometabolic diseases
- Injury to subcutaneous adipose tissue (SAT) is thought to promote greater storage in visceral adipose tissue (VAT)
- However, the cellular mechanisms that link SAT dysfunction with VAT expansion in PWH remain unknown

METHODS

- We performed single-cell RNA sequencing (scRNA-seq) on abdominal SAT biopsies from 44 individuals with long-term virologic suppression and a spectrum of metabolic fitness (**Fig. 1**)
- We performed partial Spearman's correlation between cell proportions and morphometric CT measurements adjusted for age, sex, body mass index (BMI), and diabetes status
- Bulk RNA sequencing was performed on 91 individuals from the same cohort
- DESeq2 was used to evaluate differentially expressed genes between those with high VAT volume (top 1/3) and low VAT volume (bottom 1/3) adjusted for age, sex, BMI, and batch.
- Gene Set Enrichment Analysis (GSEA) was used to identify significant pathway enrichment

Figure 1. Overview of Study Design



RESULTS

- Median age was 52 years, BMI 32.0 kg/m^2, and 70% were male (**Table 1**)
- VAT volume was associated with the proportions of SAT intermediate macrophages, myofibroblasts, and MYOC+ fibroblasts in scRNA-seq **(Fig 2.)**
- Those with higher VAT volume had higher expression of SAT chemokines (*CCL2, CCL3, CCL19, CCL21*), interleukins (*IL33, IL34, IL10)*, and extracellular matrix genes (*FN1, TNC, MMP9),* and reduced expression of adipogenic genes (*PPARG, ADIPOQ, FASN, KLF15, LPL*) (**Fig. 3**)
- GSEA showed enrichment of pathways related to leukocyte/monocyte migration, macrophage activation, and reduced enrichment for pathways related to fatty acid degradation, branched chain amino acid degradation, and insulin signaling (**Fig. 4)**

Table 1. Cohort Characteristics

	No Diabetes (N = 13)	Prediabetes (N = 13)	Diabetes (N = 18)	P-value
Age, median years	47 (45, 53)	51 (41, 57)	53 (47, 63)	0.53
Female (%)	5 (38)	3 (23)	5 (28)	0.68
Race, Caucasian (%)	7 (54)	6 (46)	8 (44)	0.87
BMI, kg/m^2	30.5 (29.1, 34.9)	31.4 (28.9, 35.8)	33.2 (30.5, 38.0)	0.26
INSTI-based regimen (%)	9 (82)	8 (80)	10 (71)	0.80
Older NRTI exposure (%)	5 (38)	1 (8)	2 (12)	0.10
Hemoglobin A1c, %	5.3 (5.3, 5.4)	5.7 (5.4, 6.0)	7.2 (6.3, 9.4)	**< 0.001**
Fasting glucose, mg/dL	87 (86, 91)	111 (102, 123)	184 (126, 238)	**< 0.001**

Continuous variables are shown as median value with interquartile range (parentheses).
BMI = body mass index; INSTI = integrase strand transfer inhibitor; NRTI = nucleoside reverse transcriptase inhibitor

Figure 2. Heatmap of partial Spearman's correlation between single-cell proportion and CT morphometric measurements. *P < 0.05, **P < 0.001.
Abbreviations: FIB, fibroblast; IM, intermediate macrophage; LAM, lipid-associated macrophage; Mo-Mac, monocyte-macrophage; PVM, perivascular macrophage; TEM, effector memory.

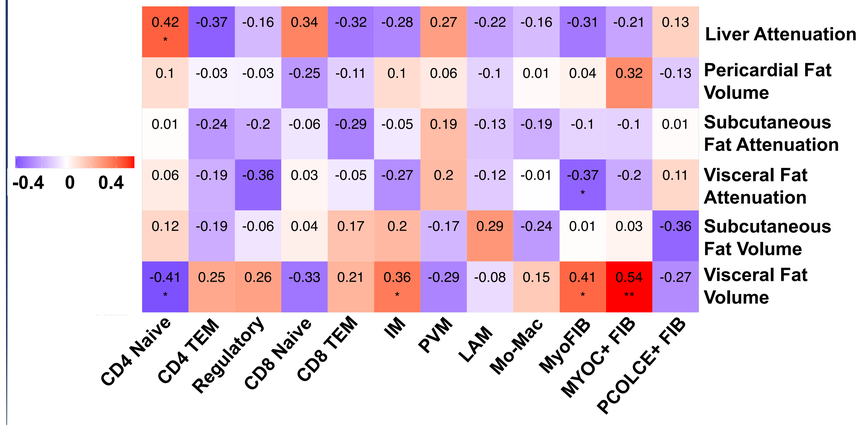


Figure 3. Volcano plot showing high vs low VAT volume using bulk RNA sequencing with average $\log_2$ fold change on the x-axis and –$\log_{10}$ adjusted p-value on the y axis. Genes with FDR-adjusted p-value < 0.05 are shown in red (increased) and blue (decreased).

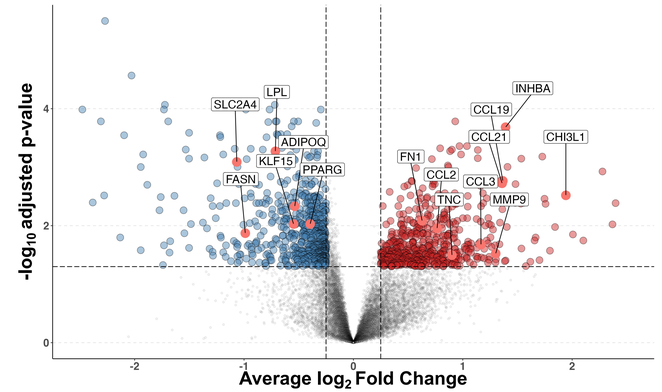
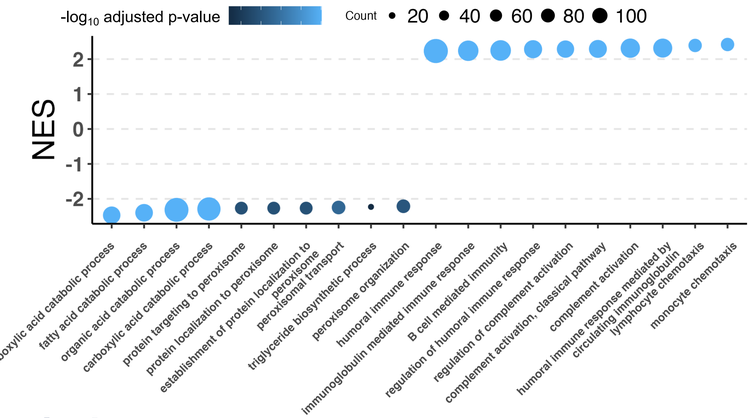


Figure 4. Gene Ontology Gene Set Enrichment Analysis. The top over- and under-represented pathways using bulk RNA sequencing comparing high vs low VAT volume. The size of the circle represents the number of genes in the pathway and the color represents the –$\log_{10}$ adjusted p-value. Abbreviation: NES, normalized enrichment score.



Conclusions

- We demonstrate that SAT cellular composition is associated with VAT but not SAT volume, which is driven by fibroblast and inflammatory macrophage cells that influence overall SAT gene expression
- Future studies evaluating the longitudinal relationship of these cell types with VAT volume are necessary to investigate causal relationships and underlying mechanisms

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