# Mechanical and Histological Properties of Healthy and Unhealthy Human Adipose Tissue Extracellular Matrix

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## INTRODUCTION

Dysregulation of adipose tissue extracellular matrix (ECM) deposition, a.k.a. fibrosis, is a known feature of obesity, but the precise role of adipose ECM in the pathogenesis of metabolic disease is unknown. Current dogma suggests that increased ECM is associated with unhealthy adipose tissue[1]; however, data supporting this hypothesis is conflicting as some studies demonstrate an increase in ECM is associated with healthy adipose tissue[2], while others demonstrate the opposite. Furthermore, while different methods are used to quantify tissue ECM, correlations of these metrics with metabolic disease and with one another are not well-described. Our goal here was to determine the correlation of ECM deposition in adipose tissue with metabolic disease in a well-characterized cohort of humans with obesity. To accomplish this, we investigated dysregulation of human adipose tissue ECM with transcriptomics, tissue rheology, and histologic immunohistochemistry.

***<u>Hypothesis:</u>*** Adipose tissue ECM gene expression, mechanical stiffness, and histologic collagen deposition will be <u>increased</u> in patients with metabolic disease and <u>increased</u> in visceral compared to subcutaneous adipose tissue (VAT vs. SAT), given VAT's stronger association with metabolic disease[3].

## METHODS

Human VAT and SAT samples collected during surgical procedures from humans with obesity were studied with RNA Sequencing, macro-rheologic compression testing, and histologic analysis.

**Transcriptomics**

- Total RNA extracted, subjected to bulk RNASeq. Pathway analysis performed with iPathwayGuide Advaita, Inc. Differentially expressed genes (DEG) identified using thresholds p-value < 0.05, logFC > 0.5.

**Static Rheology**

- 6mm punch biopsies of adipose tissue were studied with macro-rheology using HR30 Discovery Hybrid Rheometer (TA Instruments) to determine Young's modulus.

**Histology**

- 6µm sectioned adipose tissue samples were stained with Picrosirius Red, imaged, and digitally processed with Aperio Software to quantify ECM deposition.

Data analyzed with multivariate mixed effects model.

## RESULTS

### ECM gene ontology pathways are dysregulated in the context of depot, diabetic status, and sex

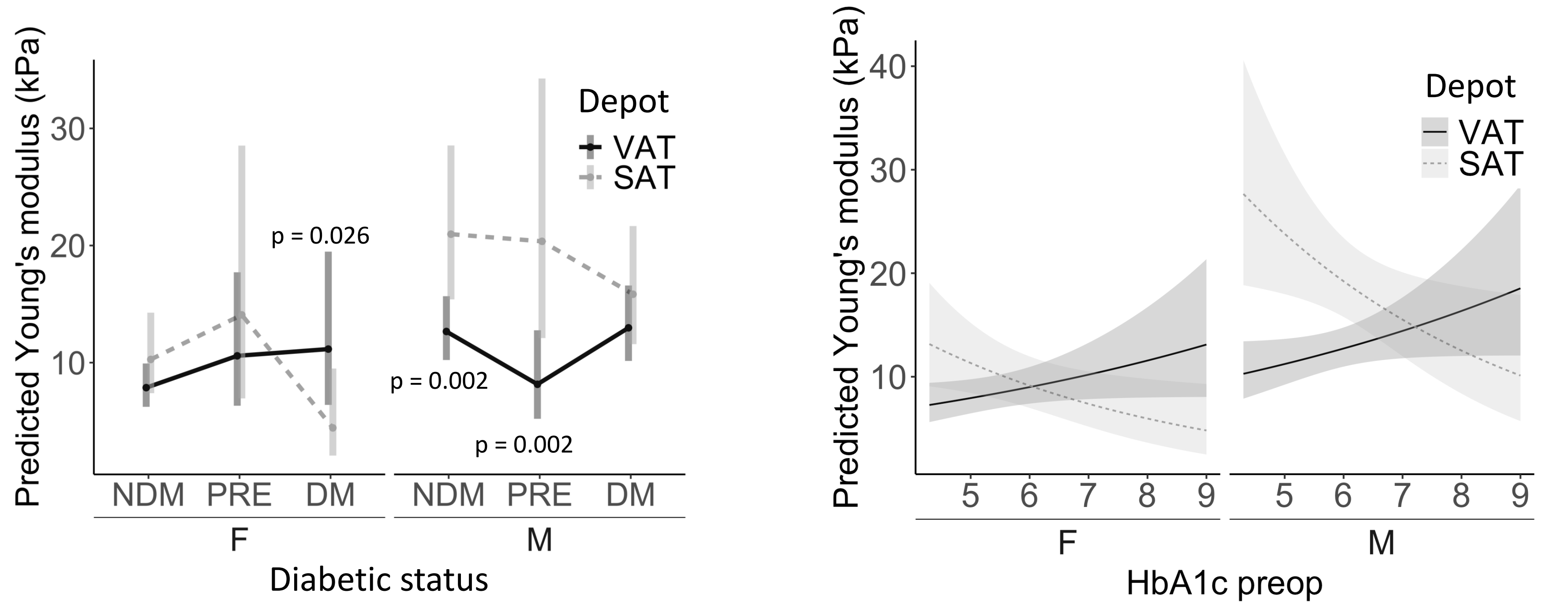
RNASeq results were analyzed using iPathwayGuide$^{TM}$ (Advaita Corp., Ann Arbor, MI, USA) which calculates dysregulation of pathways/gene sets accounting for both classical over-representation (enrichment) analysis of DEG and total pathway perturbation accumulation/topology. Pathway dysregulation was compared between VAT vs. SAT, diabetic (DM) vs. non-diabetic (NDM), and male vs. female, with four subgroups in each comparison defined by remaining variables. ECM gene ontology (GO) cellular component dysregulation ranked among the top significantly dysregulated GO cellular component gene sets in the context of depot, diabetic status, and sex.

| **VAT vs SAT** | **Diabetic Male** | | | **Diabetic Female** | | | **Nondiabetic Male** | | | **Nondiabetic Female** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathway** | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR |
| Extracellular region (GO:0005576) | 483 / 3309 | 2nd out of 153 | 1.000e-24 | 699 / 3328 | 2nd out of 163 | 1.000e-24 | 487 / 3338 | 4th out of 166 | 1.000e-24 | 809 / 3330 | 2nd out of 191 | 1.000e-24 |
| Extracellular space (GO:0005615) | 404 / 2687 | 3rd out of 153 | 1.000e-24 | 559 / 2698 | 6th out of 163 | 1.000e-24 | 392 / 2704 | 7th out of 166 | 1.000e-24 | 644 / 2694 | 6th out of 191 | 1.000e-24 |
| Extracellular matrix (GO:0031012) | 130 / 453 | 6th out of 153 | 1.000e-24 | 195 / 458 | 4th out of 163 | 1.000e-24 | 146 / 449 | 3rd out of 166 | 1.000e-24 | 202 / 455 | 4th out of 191 | 1.000e-24 |

| **DM vs NDM** | **Male VAT** | | | **Male SAT** | | | **Female VAT** | | | **Female SAT** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathway** | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR |
| Extracellular region (GO:0005576) | 84 / 3291 | 2nd out of 19 | 9.882e-12 | 240 / 3310 | 3rd out of 152 | 2.769e-23 | 173 / 3323 | 1st out of 89 | 1.000e-24 | 229 / 3234 | 1st out of 81 | 8.736e-23 |
| Extracellular space (GO:0005615) | 65 / 2671 | 6th out of 19 | 1.342e-7 | 192 / 2689 | 7th out of 152 | 8.581e-17 | 132 / 2691 | 7th out of 89 | 7.983e-17 | 197 / 2628 | 2nd out of 81 | 8.736e-22 |
| Extracellular matrix (GO:0031012) | 29 / 447 | 1st out of 19 | 9.882e-12 | 56 / 449 | 9th out of 152 | 7.810e-13 | 38 / 451 | 15th out of 89 | 2.472e-10 | 52 / 437 | 5th out of 81 | 1.747e-11 |

| **Male vs Female** | **Diabetic VAT** | | | **Diabetic SAT** | | | **Nondiabetic VAT** | | | **Nondiabetic SAT** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pathway** | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR | #DEG/ ALL | Rank in sig. dysregulated gene sets, FDR | p-value, FDR |
| Extracellular region (GO:0005576) | 180 / 3294 | 2nd out of 74 | 1.705e-23 | 293 / 3292 | 2nd out of 111 | 1.000e-24 | 100 / 3316 | 1st out of 24 | 7.200e-13 | 251 / 3269 | 5th out of 104 | 2.952e-18 |
| Extracellular space (GO:0005615) | 140 / 2670 | 6th out of 74 | 1.957e-15 | 227 / 2674 | 6th out of 111 | 3.315e-20 | 79 / 2687 | 2nd out of 24 | 8.325e-9 | 213 / 2656 | 7th out of 104 | 5.176e-17 |
| Extracellular matrix (GO:0031012) | 58 / 450 | 3rd out of 74 | 5.590e-22 | 84 / 450 | 4th out of 111 | 1.000e-24 | 25 / 447 | 5th out of 24 | 4.436e-7 | 64 / 441 | 10th out of 104 | 5.301e-15 |

### Adipose tissue mechanics are regulated by depot, diabetic status, and sex

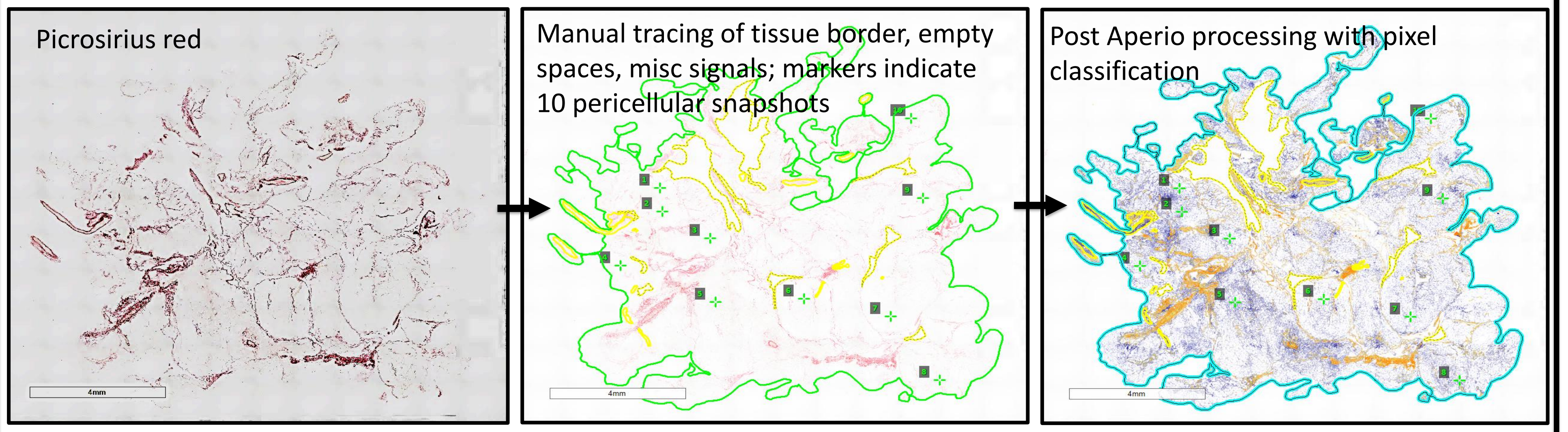


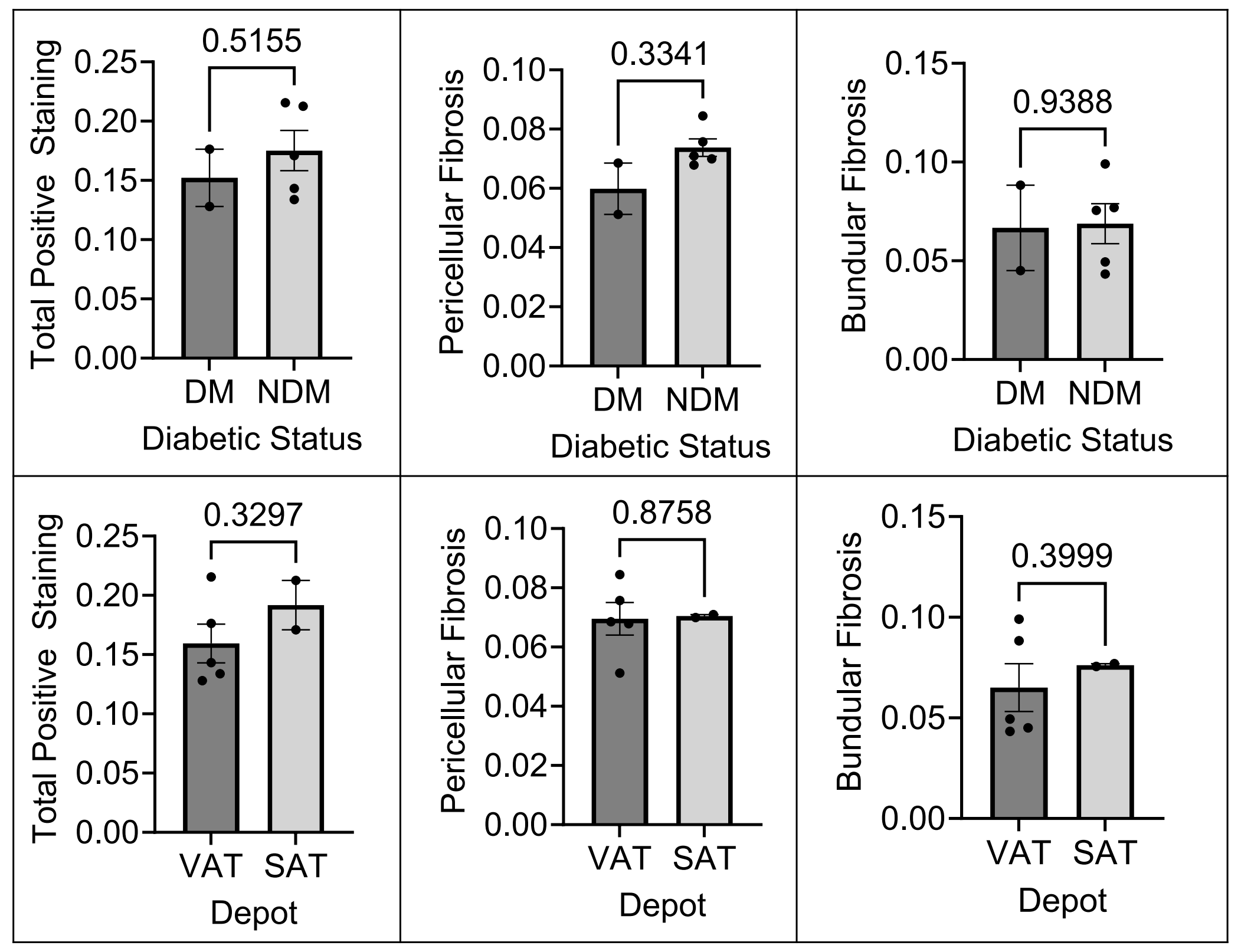
Mechanical properties of human adipose tissue studied with static compression rheology revealed:

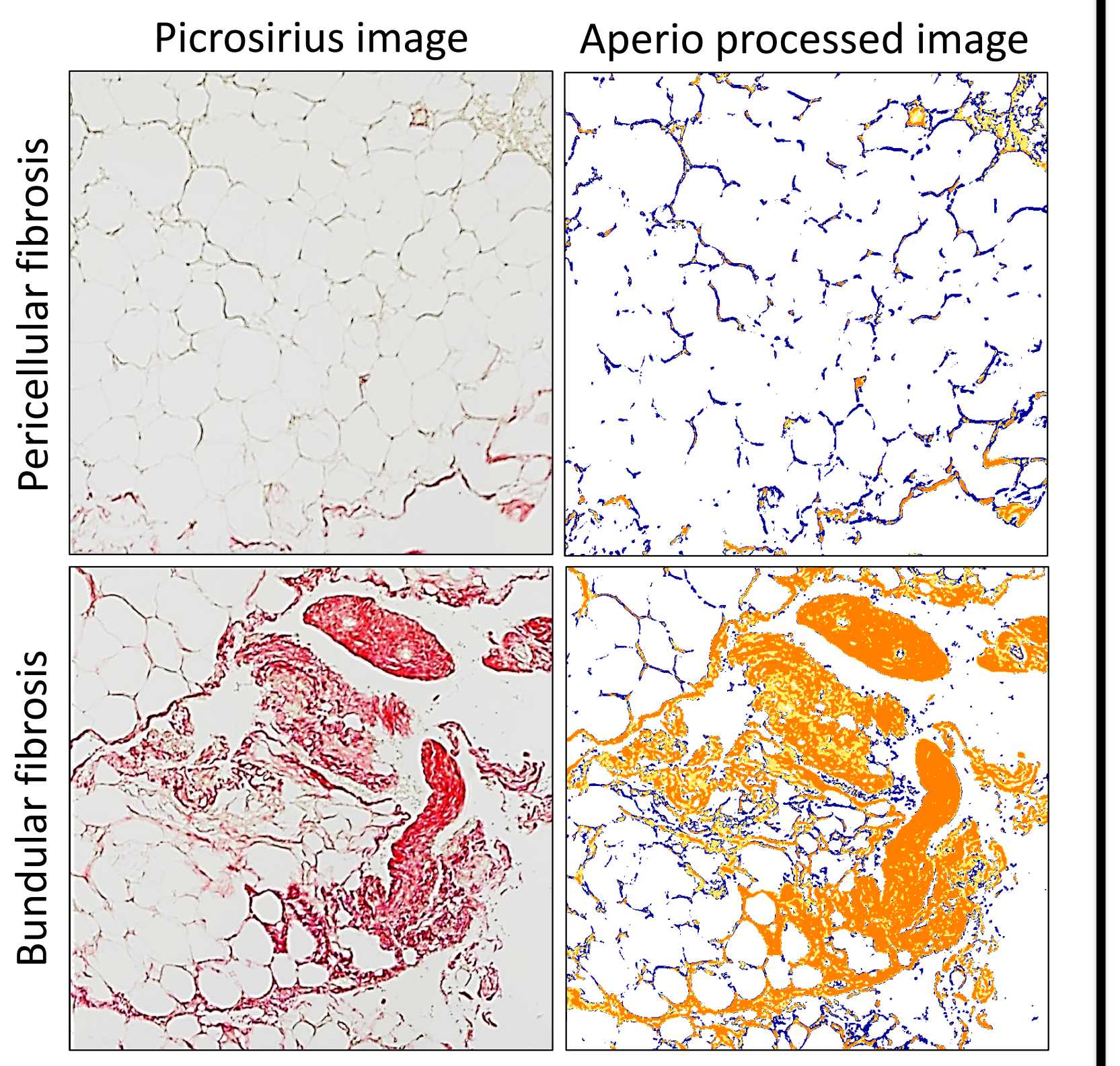
<u>Left</u>: Adipose tissues from males are generally stiffer than tissues from females; furthermore, SAT is stiffer than VAT in NDM males, but this difference disappears in DM males due to a decreasing trend in SAT stiffness with progression to diabetes.

<u>Right</u>: Similar trends were observed when HbA1c was studied as a continuous dependent variable of diabetic status, such that VAT stiffness increased and SAT stiffness decreased with disease progression.

### Development of a method for digital quantitation of immunohistochemistry of adipose tissue ECM







To facilitate histologic analysis of adipose tissue fibrosis, we developed a method for digital quantitation of collagen in Sirius Red-stained fixed adipose tissue sections using Aperio Software (Aperio Software Inc.), permitting quantitation of pericellular, bundular, and total fibrosis. Initial pilot analysis demonstrates feasibility of this method. No correlation was observed between sex, depot or DM status and quantity or type of ECM deposition (data shown for NDM vs DM and VAT vs SAT only), although this may be influenced by the small sample size (n=7) and underpowered.

## CONCLUSIONS

- Dysregulation of ECM gene pathways in adipose tissue is a central feature of adipose tissue in obesity, and is regulated by differences in depot, diabetic status, and sex.
- Contrary to our initial hypothesis and current dogma, adipose tissue stiffness does not always correlate with metabolic diseases.
- Specifically, subcutaneous adipose tissue is stiffer than visceral adipose tissue in nondiabetics and subcutaneous adipose tissue stiffness decreases with disease progression in males.
- These observations will inform design of future studies, which will require inclusion of tissues from multiple depots and both sexes.
- These findings support the novel concept that increased adipose tissue ECM may, in some contexts, be an indicator of metabolic health and robust tissue remodeling capacity.

## REFERENCES

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3. Chang E, Varghese M, Singer K. Gender and Sex Differences in Adipose Tissue. *Curr Diab Rep*. 2018;18(9):69.