

Depot identity drives functional and transcriptional disparities between subcutaneous and visceral adipose tissue

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Introduction

In the LiMa Project study¹ we demonstrated that mouse visceral adipose tissue (vWAT), retains a persistent mitochondrial and redox “metabolic imprint” after obesity, even when several phenotypical parameters normalize after a lifestyle intervention. This imprinting includes sustained alterations in mitochondrial respiration, redox balance, and transcriptional programs, suggesting that prior nutritional stress leaves molecular memory². Importantly, this phenomenon was specifically defined in the vWAT, but not in subcutaneous adipose tissue (scWAT) depot, raising the possibility that adipose depots differ fundamentally in their long-term vulnerability to metabolic overload. In addition, further insights from the literature also evidence that these inter-depot differences are also manifested in the tight interplay between obesity progression and degenerative aging processes³.

These potential differences could be due to anatomical location and functional specificity. scWAT is generally described as a metabolically flexible depot capable of expanding through adipogenesis while maintaining insulin sensitivity and exhibiting a relatively low inflammatory profile. scWAT is also thought to undergo limited fibrotic remodeling under metabolic stress, preserving extracellular matrix integrity and structural plasticity⁴. In contrast, vWAT is classically characterized by distinct developmental origins, enriched vascular and immune cell networks, and heightened endocrine and immunometabolic activity. vWAT is frequently associated with increased oxidative stress, mitochondrial dysfunction, and pro-inflammatory signaling during metabolic overload⁴.

In this context, the comparison between both WAT depots becomes essential. Evaluating the vWAT against the scWAT within the same model allows us to determine whether the persistent metabolic imprinting previously described in visceral fat extends beyond this depot or reflects a unique vulnerability of vWAT. To address this, we examine both tissues through the relationship between body mass and depot mass, histological assessment, mitochondrial DNA quantification, high-resolution respirometry (HRR) and RNA-seq analysis. These complementary approaches provide the basis for the results presented below, where we characterize how chronic overfeeding shapes the metabolic trajectory of each adipose depot.

Methods

Experiments were conducted on young adult C57BL/6J OladHSD male mice, maintained under standard housing conditions and assigned to a chow-fed control group (CTRL) or a high-fat diet (HFD) group for 16 weeks, generating lean and obese animals respectively¹. Comparative analyses were performed across control, HFD, and intervention groups to evaluate obesity-induced alterations and their potential reversibility.

Histological examination was done using 4 µm thick sections from formalin-fixed paraffin-embedded tissue samples, which were stained with hematoxylin and eosin (H&E)¹.

Mitochondrial DNA (mtDNA) content was determined by qPCR of total DNA extracted, using the following primers; cytochrome C oxidase subunit 2 (*mt-Co2* Fw: CTACAAGACGCCACAT – *mt-Co2* Rv: GAGAGGGGAGAGCAAT), 12S rRNA (*Mt-Rnr1* Fw: CTTCAAGCAAACCCTAAAAAGG – *Mt-Rnr1* Rv: GCGTACTTCATTGCTCAATTC) and succinate dehydrogenase subunit A (*Sdha* Fw: TACTACAGCCCCAAGTCT – *Sdha* Rv: TGGACCCATCTTCTATGC)¹.

For high resolution respirometry (HRR), both adipose depots were excised and immediately transferred into MiR05 respirometry medium. Fresh samples were weighed, mechanically homogenized (40 mg/mL), and chemically permeabilized with digitonin (8,1 mM). Permeabilized homogenates (2 mL) were then loaded into O2k chambers and the substrate-uncoupler-inhibitor titration protocol SUIT-008 (<https://wiki.oroboros.at/index.php/SUIT-008>) was used for allowing sequential evaluation of leak respiration, OXPHOS capacity, electron transfer (ET) capacity, and residual oxygen consumption (ROX). Oxygen fluxes were corrected for instrumental background and ROX and normalized to tissue mass.

RNA samples from vWAT (Ctrl n = 4, HFD n = 8) and scWAT (Ctrl n = 4, HFD n = 4) were sequenced using standard Illumina protocols. Differential expression analysis was performed with the DESeq2 package and GO term enrichment was assessed using GOstats for biological processes and cellular components (p < 0.0001). To evaluate coordinated transcriptional changes, we additionally conducted GSEA using preranked gene lists and curated mitochondrial gene sets from MitoCarta.

Results

Inter-depot differences in tissue mass evolution. The impact of HFD feeding:

Linear regression analyses between body mass and adipose tissue mass revealed distinct patterns between depots. In scWAT, adipose tissue mass was positively correlated with body mass in both CTRL and HFD groups. In contrast, vWAT exhibited a positive correlation with body mass in control animals but a negative correlation in the HFD group.

HFD promotes adipocyte hypertrophy and inflammatory remodeling in vWAT but not in scWAT:

Hematoxylin and eosin staining revealed differences in adipose tissue morphology between depots. In vWAT, the HFD group exhibited crown-like structures, whereas these were largely absent in the CTRL group. In contrast, both CTRL and HFD animals displayed a similar histological appearance in scWAT, with no evident immune cell infiltration, crown-like structures, or fibrosis. Adipocyte size distribution analysis revealed hypertrophic remodelling in both adipose depots in response to HFD. In CTRLs, the highest frequency of adipocytes was found within the 100-200 μm^2 area interval in both vWAT and scWAT. In contrast, HFD intervention shifted the distribution towards larger cell sizes, with the most frequent adipocyte area corresponding to the 400-800 μm^2 interval in vWAT and the 200-400 μm^2 interval in scWAT.

Mitochondrial content is reduced in vWAT but preserved in scWAT following HFD:

Analysis of mtDNA copy number revealed depot-specific alterations in response to HFD. A marked reduction in mtDNA copy number was observed in vWAT from HFD animals compared with controls, indicating a decrease in mitochondrial content within this depot. In contrast, mtDNA copy number in scWAT remained unchanged between groups.

Visceral and subcutaneous adipose tissue exhibit distinct respiratory responses to HFD:

HRR analyses revealed depot-specific differences in mitochondrial respiratory function. In vWAT, HFD induced a reduction in respiratory capacity compared with control animals, indicating an impairment of mitochondrial function in this depot. In contrast, no significant differences in oxygen flux were detected between CTRL and HFD groups in scWAT across the respiratory states analyzed: OXPHOS NADH- and succinate-linked pathways activated: CTRL vWAT: 3.52 ± 1.12 , HFD vWAT: 1.81 ± 0.59 CTRL scWAT: 2.01 ± 0.20 , HFD vWAT: 1.31 ± 0.24 $\text{pmol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$. Furthermore, absolute O_2 flux values were consistently higher in vWAT than in scWAT in both experimental groups, reflecting a greater overall respiratory activity in the visceral depot.

Depot-specific transcriptional alterations in response to HFD:

RNA-seq analysis reveals markedly distinct transcriptional responses between the two adipose depots when challenged with a HFD. In vWAT, the response to HFD is characterized by a profound downregulation of predominantly mitochondrial pathways, negatively impacting oxidative phosphorylation, the electron transport system, and cellular respiration. Concurrently, vWAT undergoes a strong upregulation of processes linked

to immune activation, inflammatory response and extracellular matrix organization. Conversely, scWAT exhibits an opposing dynamic: overall, it lacks notable mitochondrial alterations among its repressed genes, with its downregulation being primarily linked to immunity and RNA processing. In fact, unlike vWAT, mitochondrial modifications in scWAT are observed within upregulated pathways, which include mitochondrial organization and the response to oxidative stress, alongside an increase in vesicle localization and transport.

Discussion

The results of this comparative approach demonstrate a markedly divergent metabolic and structural response between vWAT and scWAT to a HFD intervention. While scWAT exhibits a physiological and continuous expansion in relation to body mass gain, vWAT displays a clear limitation in its expandability. The inability of vWAT to safely accommodate excess lipids translates into severe pathological hypertrophy, reaching bigger cellular areas. Morphologically, this mechanical and cellular stress triggers an inflammatory remodeling confirmed by the appearance of crown-like structures, which are completely absent in scWAT.

At cellular level, vWAT dysfunction is intimately linked to a consistent mitochondrial impairment. HFD feeding causes a drastic reduction in mtDNA copy number and a significant decline in respiratory capacity within this depot, even though vWAT exposed a higher oxygen consumption per mass compared with its subcutaneous counterpart. This functional failure is strongly supported by the transcriptomic data, which reveals a massive repression of essential pathways for oxidative phosphorylation and the electron transport system. Simultaneously, the expression profile of vWAT confirms the nature of its tissue failure through the robust upregulation of immune and proinflammatory pathways.

In contrast, scWAT demonstrates remarkable metabolic resilience. Despite undergoing moderate hypertrophy, this depot successfully preserves its mitochondrial network and respiratory function. The transcriptional response of scWAT suggests an active and protective adaptation: instead of depressing its metabolism, it upregulates genes related to mitochondrial organization, vesicle transport, and defense against oxidative stress. The concurrent repression of immune pathways in scWAT indicates that this tissue can expand without triggering local inflammation.

Altogether, these findings support the adipose tissue expandability paradigm. Upon reaching its physical and metabolic threshold, vWAT undergoes a decompensation characterized by severe mitochondrial dysregulation, inflammation and extracellular matrix organization. Conversely, scWAT acts as an efficient lipid sink, activating mitochondrial compensatory mechanisms that prevent immune cell recruitment and lipotoxicity, thereby highlighting the critical role of anatomical location and functional specificity in adipose tissue plasticity.

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