Characterization of Adipose Depot Tissue Transcriptome in Response to Sex and Aging

Hunter Hasskamp[1], Stephen A. Martin[1]
[1]Montana State University, Bozeman, MT
hunterhasskamp@montana.edu

Disclosures: Hunter Hasskamp (N), Stephen A. Martin (N)

INTRODUCTION: Bone marrow adipose tissue (BMAT) is an endocrine tissue that influences skeletal health and systemic physiology through the secretion of adipokines, hormones, metabolites, and lipids. In healthy individuals, BMAT quantity typically shows an inverse relationship with bone mineral density; osteoporotic and older adults exhibit greater marrow adiposity than younger, healthy counterparts. While the adverse effects of aging on skeletal and metabolic health are well documented, how aging alters BMAT phenotype, and whether the marrow microenvironment influences bone properties, remains largely unknown. This study aimed to characterize how age and sex independently and interactively influence the BMAT transcriptome in F344xBN F1 hybrid rats, and how these responses overlap with or diverge from transcriptomic signatures in extramedullary visceral (VAT) and subcutaneous adipose tissue (SAT). We hypothesize that aging drives broad transcriptomic alterations in BMAT, occurring in a sexually dimorphic fashion and distinct from VAT and SAT. By investigating these age- and sex-related changes, we aim to identify targets for interventions promote healthy aging and uncover mechanisms linking BMAT to skeletal properties.

METHODS: All animal procedures were approved by the Montana State University IACUC. Female and male F344xBN F1 hybrid rat (7, 15, and 22 months; n = 8 per group) were obtained from the National Institute on Aging Aged Rodent Colony. The animals maintained Purina Mills 5L79 chow (13.7% fat) ad libitum under a standard light cycle. Following euthanasia by cardiac puncture and exsanguination, inguinal SAT and reproductive VAT, and distal tibia BMAT (collected by centrifugation) were harvested and stored at -80□C. In adult rats, the distal tibial marrow predominantly comprised of adipocytes. RNA was extracted using RNeasy Lipid Kits (Qiagen), and bulk RNA sequencing was performed on an Illuminex NovaSeq 6000 platform. Transcript counts were analyzed utilizing principal component analysis, differential expression, over-representation analysis, gene set enrichment analysis, gene coexpression network analysis, and regulatory network analysis. Differentially regulated genes were defined by a |log2(Fold Change) | > 1 with adjusted p-value less than 0.05 (negative Binomial GLM, Wald test, FDR correction).

RESULTS SECTION: BMAT exhibited distinct transcriptomic signatures compared to VAT and SAT. Across depot comparisons, 31 pathways were negatively enriched and 20 positively enriched in BMAT. Additional depot-specific differences included 37 negative and 10 positive pathways for BMAT vs. VAT, and 11 negative and 7 positive pathways for BMAT vs. SAT, highlighting BMAT's unique phenotype compared to extramedullary depots. Aging from 7 to 22 months in BMAT was associated with only three enriched pathways (1 negative and 2 positive), indicating modest but biologically relevant age-related remodeling. In contrast, sex exerted a strong effect on the BMAT transcriptome: male BMAT displayed 58 negatively and 2 positively enriched pathways relative to females. Age × sex interactions contributed three additional pathways (1 negative and 2 positive). Gene coexpression network analysis identified 10 modules associated with experimental factors. Hub genes included Lamc1, Aplp2, and Erc1 (Steel blue); Znf865, Mysm1, and Vps26c (Green); and Rps15, Rps7, and Rpl27a (Light green). Notably, Lamc1 promotes osteogenesis and suppresses adipogenesis, suggesting functional relevance to BMAT–bone interactions. Regulatory network analysis further identified Umps, Mxd1, Stat6, Tcf3, and Zfp251 as highly connected regulators involved in growth, differentiation, immune signaling, and homeostasis, pointing to potential mechanisms by which systemic signals are integrated within the marrow environment.

DISCUSSION: This study demonstrates that BMAT exhibits a transcriptional signature distinct from VAT and SAT, suggesting functional differences among adipose depots. Sex, more than age or age × sex interactions, was the dominant driver of transcriptomic variation, with differences most evident in signaling and metabolic pathways, indicating both systemic endocrine and local metabolic consequences. Aging was linked primarily to cytoskeletal and adhesion pathways, while age × sex interactions influenced cytokine signaling and ribosomal/tRNA biosynthesis, suggesting sex-dependent modulation of inflammatory and translational processes. Network analyses further refined these insights. The Steel blue module, enriched in extracellular matrix and vesicle trafficking genes, correlated negatively with BMAT but positively with VAT, with Lamc1 emerging as a hub gene linking BMAT biology to skeletal outcomes. The Green module, associated with transcriptional and membrane-transport programs, was suppressed in BMAT compared to peripheral depots. The Light green module, linked to ribosomal biogenesis, showed sex-dependent correlations, suggesting translational control mechanisms that may underlie sex-biased skeletal fragility. Regulatory network analysis identified transcription factors (Umps, Mxd1, Stat6, Tcf3, Zfp251) with broad roles in differentiation, growth, and immune regulation, indicating that BMAT transcriptional programs are governed by pathways central to cellular development and immune–metabolic interactions. These regulatory signatures highlight BMAT's capacity to integrate local bone marrow signals with systemic metabolic regulation, reinforcing its role as a likely dynamic endocrine component of the marrow niche. By connecting BMAT biology to processes involved in tissue remodeling and inflammatory signaling, these findings suggest that BMAT may represent a key determinant of skeletal integrity and a potential target for interventions aimed at reducing age- and sex-related bone fragility. Although bulk RNA-seq limits resolution at the cellular level, these findings nominate candidate pathways and regulatory factors for future mechanistic investigation. In summary, BMAT is transcriptionally and functionally distinct from extramedullary adipose depots, with sex emerging as a principal determinant of phenotype. These results highlight BMAT's potential role in shaping age- and sex-specific skeletal outcomes and underscore its importance as a therapeutic target for preserving bone health. Collectively, this work advances the conceptual framework that BMAT functions as a unique, bone-coupled adipose depot with distinct contributions to skeletal integrity across the lifespan.

SIGNIFICANCE/CLINICAL RELEVANCE: This work advances understanding of the bone marrow niche by delineating functional differences among BMAT, VAT, and SAT. These insights provide a foundation for identifying mechanisms regulating BMAT and bone crosstalk and for developing interventions that modulate this relationship. Such strategies hold promise for extending healthspan in an aging population, with a precision medicine approach that accounts for sex-specific with differences.