

# Mitochondrial Dysfunction is Associated with Infrapatellar Fat Pad Fibrosis in Human and Murine Knee Osteoarthritis

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**INTRODUCTION:** Tissue fibrosis is commonly observed in patients with knee osteoarthritis (OA), associated with pain and stiffness<sup>1</sup>. Increasing evidence indicates that the infrapatellar fat pad (IPFP) is a significant contributor to the fibrotic component of OA<sup>2</sup>. Despite its high prevalence and clinical impact, we currently lack efficacious therapies to treat joint fibrosis, due in part to our limited understanding of the molecular basis underlying this pathological process. Dysfunction of the mitochondria, which are key regulators of metabolism, apoptosis, and homeostasis, has been implicated in organ fibrosis<sup>3</sup>. However, the specific role of mitochondrial dysfunction in joint fibrosis remains largely unexplored. In this study, we integrated gene expression analyses of clinical samples and mouse models aiming to identify evidence of mitochondrial dysfunction associated with joint fibrosis in OA.

**METHODS: Tissue retrieval:** IPFP tissue samples were collected at the time of surgery from patients undergoing total knee arthroplasty for OA (N=9) or anterior cruciate ligament reconstruction (ACLR, N=9), with IRB approval and patient consent. **Mouse tibial loading:** After IACUC approval, 11-to-12-week-old C57BL/6J male mice were subjected to daily tibial loading to induce joint fibrosis and OA pathology, as described<sup>4</sup>. Range of motion was assessed at 1 and 2 weeks after initiation of loading<sup>5</sup>. At 2 weeks after initiation of loading, IPFPs from loaded and unloaded contralateral hindlimbs (control, ctrl) were processed for histology (N=3/ea) and RNA isolation using TRIzol followed by purification using the Qiagen RNeasy kit (N=4/ea). **Histology:** 7µm formalin-fixed and paraffin-embedded sections were used for Masson's Trichrome or H&E staining and histological fibrosis scoring<sup>4</sup>. **RNA-seq:** A total of 100ng of RNA from human or mouse IPFPs were used for RNA-seq at the Genomics Resources Core Facility of Weill Cornell Medicine. After sequencing the reads were processed at the HSS Genomics Research Center following established pipelines<sup>6</sup>. **RTqPCR:** Amplifications were carried out using the QuantStudio™ 3 Real-Time PCR System and Fast SYBR™ Green Master Mix (ThermoFisher Scientific), and mouse-specific primers against Atp5pb (ATP synthase F1 subunit alpha), Sod2 (superoxide dismutase 2), Opa1 (OPA1 mitochondrial dynamin-like GTPase), Cox8b (cytochrome c oxidase subunit 8B), Pink1 (PTEN induced kinase 1), and Ppargc1a (PPARG coactivator 1 alpha). The data were calculated as the ratio of each gene to Eef1a1.

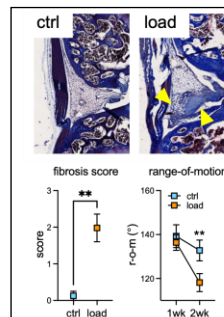
**RESULTS:** Compared to samples from ACLR patients, samples from OA patients displayed increased expression of fibrosis-associated genes by RNA-seq. Conversely, markers of mitochondrial function were downregulated in patients with OA vs. ACLR samples. QuSAGE pathway analyses revealed significant downregulation of genes encoding key enzymes in the tricarboxylic acid (TCA) cycle, including aconitase-1, malate dehydrogenase-1, and isocitrate dehydrogenase-1. Similarly, fatty acid β-oxidation and electron transport chain (ETC) pathways were decreased in OA. In agreement with previous reports, mouse tibial loading was associated with fibrosis at 2 weeks after loading (Fig.1). The increased fibrosis was associated with reduced range-of-motion at 2 weeks after loading (Fig.1). In agreement with our findings in human samples, RNA-seq analyses of control and loaded IPFPs revealed a pronounced downregulation of genes associated with mitochondrial function (Fig.2). RTqPCR analyses of selected genes further confirmed these observations (Fig.3).

**DISCUSSION:** Integrating analyses of clinical samples and mouse models of OA-associated fibrosis, in this study we provide evidence suggesting that IPFP fibrosis in OA disease is associated with mitochondrial dysfunction. Through comprehensive gene expression analyses, we show that key regulatory pathways within the mitochondria are affected in the setting of joint fibrosis. Specifically, we observed a consistent downregulation of genes associated with mitochondrial biogenesis, mitophagy, and oxidative phosphorylation in both human and mouse OA IPFPs, concomitant with increased expression of fibrosis-associated genes and histological evidence of fibrosis. Among the genes analyzed, we found that the expression of Opa1, located in the inner mitochondrial membrane and with key roles in mitochondrial stability and fusion, was significantly decreased in samples from loaded (OA) limbs compared to unloaded controls. Similarly, the expression of genes directly related to mitochondrial function and ATP production (e.g., Cox8b, Atp5pb, and Idh3) was decreased in OA vs. control samples, and other targets involved in the regulation of mitochondrial biogenesis (Ppargc1a) and protective pathways via mitophagy (Pink1) displayed very pronounced changes in gene expression (40-fold and 12-fold, respectively) comparing control and loaded limbs. While our study is limited to gene expression analyses, we identified changes consistent with mitochondrial dysfunction associated with IPFP fibrosis and OA disease, which warrant further investigation. Future directions should comprehensively integrate gene expression and histological analyses with proteomic approaches and mitochondrial functional assays.

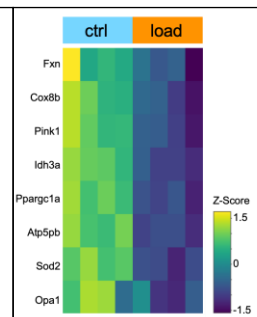
**SIGNIFICANCE/CLINICAL RELEVANCE:** Understanding the relative contribution of mitochondrial dysfunction to IPFP fibrosis in OA may provide additional insight into the development of joint fibrosis and its contribution to OA disease. Exploring the functional consequences of mitochondrial dysfunction in the context of IPFP fibrosis may ultimately pave the way for the development of targeted interventions or therapeutic strategies to mitigate or reverse the progression of this condition.

**REFERENCES:** (1) PMID: 33374529, (2) PMID: 33014539, (3) PMID: 32963808, (4) doi.org/10.1002/jor.24815, (5) PMID: 35266583, (6) PMID: 34075069

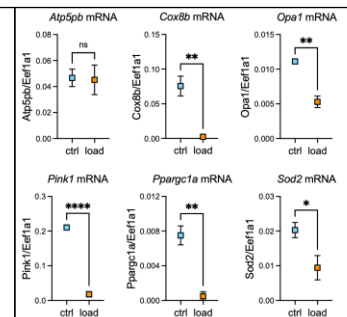
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**Figure 1:** IPFP fibrosis at 2 weeks after tibial loading (n=3/ea) is associated with decreased range of motion (n=4/a).



**Figure 2:** Decreased expression of mitochondria-associated genes in mouse IPFPs after 2 weeks of loading



**Figure 3:** Decreased expression of mitochondria-associated genes after loading analyzed by RTqPCR. \*p<0.05, \*\*p<0.01, \*\*\*p<0.0001 by t-test. (ns=not