Can/should we harmonize tissue sampling, analysis protocols, and pain measures in obesity-OA models to improve reproducibility and translatability? Miguel Otero, Purva Singh, Daniel Ramirez, Bella Mehta

RESPONSE/RECOMMENDATION: Similar to research leveraging specimens of human origin[1], research relying on mouse models of osteoarthritis (OA) is often confounded by sampling, model nuances and by the heterogeneous involvement of different joint tissues. This complexity is particularly relevant for obesity-OA models, which engage both biomechanical and biochemical mechanisms[2]. Histopathology is often used as the initial first step to evaluate OA pathology and guide downstream analyses. Thus, efforts to standardize approaches have traditionally focused on developing scoring systems to evaluate cartilage damage[3] or consolidate approaches to study the synovium in surgical models of OA[4]. We recommend that the field considers the consolidation of key aspects that enable cross-study comparisons, including selection of regions-of-interest analyzed within the joint or a given tissue, and metrics to define OA pathology (i.e., cartilage structure, osteophyte formation, synovial inflammation, fibrosis, or changes in the bone compartment). These aspects are essential to enable comprehensive multimodal analyses, improve reproducibility of studies across laboratories, and enable translational efforts.

LEVEL OF EVIDENCE: Expert Opinion

DELEGATE VOTE:

RATIONALE: Human OA is a complex multifactorial disease encompassing sex, age, genetic, traumatic, or obesity related risk factors, and affecting all joint tissues[5]. It is well accepted that we don't have a mouse model that can mimic all human OA phenotypes, and over the years researchers have developed different mouse models that recapitulate different aspects of the disease. Mouse models used to study the impact of obesity and adiposity to OA pathology can address symptomatic and structural disease, permitting the evaluation of biochemical and biomechanical factors[2]. However, different studies have relied on different readouts to assess pathology, and the field lacks a standard, harmonized approach that provides guidance of regions and features of interest, and consolidates downstream analyses for commonly used readouts, including histopathology (tissue structure and composition), genomics and targeted gene expression analyses, or pain-related outcomes. The lack of harmonization adds complexity and confounding factors, and may impede reproducibility, which is essential to translate finds to human studies. Thus, this question was motivated by the apparent lack of consensus on methods used to study obesity-related osteoarthritis (OA) in mouse models, which mirrors challenges in studies leveraging clinical material[1]. To address this question, we performed a PubMed search that identified 223 manuscripts published from 2016 to 2026, excluding preprints and focusing on other species (non-human). We searched for "obesity mouse osteoarthritis", "adiposity mouse osteoarthritis", or "high fat diet mouse osteoarthritis". For iterative searches, we included "histopathology", "genomics", "transcriptomics", "RNA-seq", "pain", or "outcomes". We did not include manuscripts that were not osteoarthritis- or obesity-specific (i.e., did not rely on OA models or did not address the impact of obesity/adiposity in OA pathology), and studies re-analyzing previously published datasets were also excluded. Due to space limitations, we only cite some of the references evaluated and focus primarily on histopathology. We did not identify manuscripts specifically addressing caveats with tissue retrieval or sampling across models; thus, we focused on studies that evaluate structural (joint histopathology) and symptomatic (pain) OA-like features, and studies leveraging genomics to study mechanisms. Most studies relying on mouse models to study the impact of obesity rely on histology to evaluate joint tissues and disease development. Several studies have relied on the modified Mankin Score to test changes in cartilage structure[6-12], while other studies have used the mouse OARSI scoring system, often with modifications and lack of consistency in the number of sections used for analyses, or the regions selected for histological scoring[11,13-20], or other approaches such as modifications of the Pritzker system[21] or evaluation of damage using osteomeasure[22]. Osteophyte formation is a structural hallmark of human OA disease, with implications in symptomatic disease, including pain. While osteophyte formation (size, and maturity) was commonly evaluated using histological approaches, the methods used were not consistent across studies[6,8,11,13,14,16,21]. Similarly, several studies addressed changes in the synovial / infrapatellar fat pad compartment, evaluating synovitis or fibrosis, but there was a lack of consistency in the features, regions, or scoring systems used across studies[6-9,11,13,15-19,21]. This lack of consistency includes the type of histological stains used, and metrics implemented to score the presence of inflammation or fibrosis in joint tissues, and has prompted recent efforts to consolidate scoring of synovial tissues when using mouse models of OA[4]. Alterations in the osteochondral

compartment are known to critically affect joint homeostasis and OA pathology, and studies using obesity-OA mouse models have incorporated histology, micro-CT or osteomeasure to evaluation of bone-related changes in OA, but are not being consistently used across studies[7,9-14,20,22,23]. Pain is the symptomatic hallmark of OA pathology and the main reason by which patients seek medical help. In obesity-OA models, there are several high-quality studies addressing pain-related outcomes combining different metrics, including grip strength, von Frey, or the use of an algometer[9,12], or evaluating changes in gene expression in the dorsal root glanglion (DRG)[12], but these methods are not implemented consistently across studies. Similarly, the advent of genomics and epigenomics methods have enabled more sophisticated analyses of molecular changes associated with structural and symptomatic and functional changes in obesity-associated OA pathology in mouse models. Different studies have analyzed joint tissues using targeted gene expression, multiplex gene expression, or more advanced genomics tools[6,12-14,24], but there's very little consistency in the tissues selected, the regions of interest analyzed, or the methodology implemented, which complicates harmonization of protocols and comparison of datasets.

Conclusion: Different groups are relying on obesity-OA models (induced by diet, changes in adiposity, and traumatic injury) to dissect signaling pathways leading to OA disease, and address symptomatic and structural changes. However, there is a wide range of analysis protocols and lack of consistency in tissue sampling, which impedes comparison across datasets. Harmonization of methodological approaches would enable comparison of datasets across models and laboratories and, we believe, would accelerate translational approaches.

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