Q12. What are the critical confounders to be considered in preclinical rodent models?

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RECOMMENDATION: *Critical confounders* are intrinsic animal characteristics and experimental variables, including but not limited to the method used to induce or model OA, age, sex, obesity/weight, and the gut microbiome. These confounders independently shape OA risk, onset, severity, and symptomatology and interact with experimental interventions, obscuring causal interpretation if not appropriately studied as independent experimental variables.

LEVEL OF EVIDENCE: Strong.

DELEGATE VOTE: Agree: Disagree: Abstain:

RATIONALE: A PubMed search was conducted using terms related to osteoarthritis, preclinical rodent models, surgical and non-surgical OA model, key biological confounders (e.g., age, sex, obesity, microbiome), and disease-relevant outcomes (e.g., cartilage, synovitis, subchondral bone changes, pain behaviors, mobility impairments), and the resulting literature was reviewed and synthesized by the authors.

MECHANISTIC OUTCOMES AND RECOMMENDATIONS FOR STUDY DESIGN:

Through a systematic evaluation of the preclinical OA literature, we identified several confounders that consistently influence disease phenotypes. If not accounted for, these variables can skew findings, undermine reproducibility, and limit translational relevance to human OA, a highly heterogeneous disease. Below, we outline the evidence supporting each confounder and provide recommendations for their consideration in future studies.

Model choice/method: Model selection is the most consequential design decision in preclinical OA research, as it defines the biological framework within which all observed mechanisms and therapeutic effects occur. Numerous surgical and non-surgical rodent OA models are commonly utilized, each capturing specific aspects of disease while introducing limitations that may confound interpretation. Common surgical models include destabilization of the medial meniscus (DMM), medial meniscal tear/transection (MMT), anterior cruciate ligament transection (ACLT), and combined ligament–meniscal injuries, which model post-traumatic OA via joint instability. However, surgical insult induces an acute inflammatory response that may not reflect chronic, non-traumatic OA in patients. Non-surgical approaches include chemically induced models (monoiodoacetate (MIA), collagenase), spontaneous age-associated OA, mechanical overloading, forced tibial compression anterior cruciate ligament rupture (ACLR), and metabolic-associated OA. The MIA model produces acute pain, but its relevance to chronic OA progression and pain mechanisms remains uncertain. In contrast, chronic overload and age-related models are more physiologically relevant but slower and more variable. These models differ in the magnitude and timing of cartilage degeneration, synovial inflammation, bone remodeling, pain, and mobility impairment, complicating comparisons and mechanistic interpretation. Studies should include uninjured age-matched controls alongside sham-surgery groups to distinguish injury-, surgery-, and disease-specific effects.

Age: Aging is the strongest risk factor for OA development in humans[1]. Age-associated processes, including cellular senescence, chronic low-grade inflammation, and cumulative joint degeneration, underlie the clinical relationship between advancing age and OA incidence and severity[1]. Small-rodent models display a spontaneous age-associated OA phenotype characterized by progressive cartilage degeneration, subchondral bone and synovial pathology, inflammation, and impairments in pain-related behaviors and mobility[2-4], supporting their utility. Beyond spontaneous disease, aging also modifies post-traumatic OA (PTOA) pathology. In both mouse and rat surgical PTOA models, aged animals develop more severe joint pathology than younger animals[5]. In the MIA chemically induced OA model, aged mice showed reduced nocifensive behavior and a blunted inflammatory response, including decreased microglial activation, relative to younger animals[6]. Together, these findings identify aging as a critical modifier of structural, inflammatory, and pain-related outcomes across OA models. Future studies should treat age as an explicit experimental variable by selecting appropriate ages at induction, incorporating longitudinal assessments to capture late-stage disease, and statistically evaluating age as an independent and interacting factor. Additionally, the temporal effects of age-related mechanisms (e.g., cellular senescence and impaired autophagy) on both pain and tissue degeneration require further investigation.

Sex: Sex is a well-established risk factor for osteoarthritis, with women exhibiting both a higher prevalence and greater pain compared to men[7, 8]. Contributing sex-linked differences in OA susceptibility and symptom severity include genomic, epigenomic, anatomical variation, sex hormone signaling, and microbiome; however, the underlying mechanisms remain incompletely understood[8, 9]. In preclinical research, female rodents have historically been underrepresented because female mice and rats have delayed or attenuated cartilage

degeneration in some PTOA models (DMM), compared to their male counterparts when assessed at equivalent time points[10, 11]. Sex-dependent differences extend beyond joint structure, as studies have demonstrated divergent pain phenotypes across models, including differences in onset, magnitude, and persistence of nociceptive behaviors, between male and female mice and rats following OA induction[12-16]. Recent mechanistic studies have further revealed sex-specific microbiota to alter OA severity, implicating pathologic interactions between sex and gut microbial composition[17]. In parallel, studies isolating the role of sex hormones revealed that androgens exacerbate OA severity, whereas ovarian hormones confer partial protection against cartilage degeneration[10]. Collectively, these findings underscore sex as a critical modifier of OA structural and pain-related phenotypes in preclinical models. Accordingly, future studies should incorporate male and female cohorts, apply sex-stratified analyses across disease endpoints, and consider sex-dependent interactions with additional confounders such as menopause[18], age, microbiome composition, and metabolic status.

Obesity/Body weight: Obesity is a major clinical risk factor for OA, contributing to both disease incidence and progression through intertwined biomechanical, metabolic, and inflammatory mechanisms[19 20]. In preclinical settings, obesity is commonly modeled through dietary manipulation, where high-fat diet (HFD) fed mice develop more severe cartilage degeneration, increased mobility impairments, and heightened pain-related behaviors compared to standard chow fed controls[21 22-24], which is incompletely explained by loading due to body mass[25]. Parallel findings have been reported in rat models of surgically induced OA, in which HFD exposure exacerbated cartilage damage and amplified systemic inflammatory responses[26, 27]. Notably, HFD-fed mice exhibit worsened pathology in both spontaneous, age-associated OA and post-traumatic OA models[28]. Mechanistic studies further implicate adipose tissue as an active contributor to OA pathogenesis, with evidence supporting leptin/complement-mediated signaling pathways that promote fat–joint tissue crosstalk and local inflammatory responses[25, 29, 30]. Collectively, these findings underscore obesity as a potent disease-modifying factor in OA, which may be sexually dimorphic[31] and can increase structural damage, pain sensitivity, and invoke novel disease mechanisms beyond increased joint loading. Accordingly, future preclinical studies should incorporate body weight, activity metrics (e.g., mobility dysfunction), and metabolic status (e.g., glucose tolerance/insulin sensitivity, adipokines leptin and adiponectin, and circulating lipids) as experimental variables, evaluate weight-mediated and weight-independent effects, and account for possible confounders introduced by dietary manipulation, including systemic inflammation, endocrine signaling, and alterations in the gut microbiome, when interpreting outcomes.

Microbiome: Clinical studies have identified associations between altered microbial signatures and OA severity, with increased detection of bacterial components (e.g., lipopolysaccharide, *Streptococcus* species) in circulating serum and knee synovial fluid correlating with worsened structural pathology and pain[32-34]. Further, human gut microbiome composition was shown to associate with joint-specific pain scores, independent of body mass index[34]. Establishing a potential causal link, preclinical rodent models have been instrumental in probing mechanisms connecting microbiome health to OA progression. Germ-free mice exhibit attenuated OA pathology following surgically induced joint destabilization[35, 36]. Mechanistic studies further demonstrate that diet-induced obesity and metabolic syndrome exacerbate OA histopathology after surgical injury compared to animals maintained on standard chow; however, selective restoration of gut microbial composition without reducing body weight significantly mitigated joint pathology and local inflammation[37]. Additional studies demonstrate that the microbiome regulates OA severity via genetic disruption of microbial-sensing pathways (e.g., TLR5 deficiency)[22], fecal microbiota transplantation[36], and oral probiotic interventions in both murine[38] and rat[39] OA models. Whole-microbiome transplantation from the MRL (OA-resistant) strain alters OA outcomes in recipient mice[40]. Together, these findings indicate microbiome perturbations modify OA through effects on systemic inflammation and metabolic state, reinforcing the concept that subsets of OA represent a systemic disorder. Future preclinical studies should control or report gut microbiome variability, animal diet, and facility-specific microbial environments when interrogating OA phenotypes.

Together, these factors constitute a working framework of major confounders currently identifiable in the OA preclinical literature, not a complete accounting. As the field advances, further sources of variability will undoubtedly emerge, emphasizing the need for transparent reporting, standardized study design, and ongoing reassessment of experimental rigor.

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