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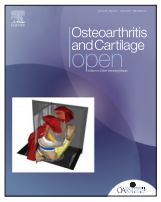

Transcriptomics and metabolomics: Challenges of studying obesity in osteoarthritis

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ABSTRACT

Objective: Obesity is a leading risk factor for both the incidence and progression of osteoarthritis (OA). Omic technologies, including transcriptomics and metabolomics are capable of identifying RNA and metabolite profiles in tissues and biofluids of OA patients. The objective of this review is to highlight studies using transcriptomics and metabolomics that contribute to our understanding of OA pathology in relation to obesity.
Design: We conducted a targeted search of PUBMED for articles, and GEO for datasets, published up to February 13, 2024, screening for those using high-throughput transcriptomic and metabolomic techniques to study human or pre-clinical animal model tissues or biofluids related to obesity-associated OA. We describe relevant studies and discuss challenges studying obesity as a disease-related factor in OA.
Results: Of the 107 publications identified by our search criteria, only 15 specifically used transcriptomics or metabolomics to study joint tissues or biofluids in obesity-related OA. Specific transcriptomic and metabolomic signatures associated with obesity-related OA have been defined in select local joint tissues, biofluids and other biological material. However, considerable challenges exist in understanding contributions of obesity-associated modifications of transcriptomes and metabolomes related to OA, including sociodemographic, anthropometric, dietary and molecular redundancy-related factors.
Conclusions: A number of additional transcriptomic and metabolomic studies are needed to comprehensively understand how obesity affects OA incidence, progression and outcomes. Integration of transcriptome and metabolome signatures from multiple tissues and biofluids, using network-based approaches will likely help to better define putative therapeutic targets that could enable precision medicine approaches to obese OA patients.

1. Introduction

Osteoarthritis (OA) is a progressive disease affecting tissues in articulating joints including articular cartilage and synovium [1]. It has been suggested that OA is a result of modified mechanical stimulation of joints, which may include increased joint loading or modified joint mechanics due to knee alignment [2]. However, recent evidence suggests that OA is not just a local joint disease, but also has contributions from systemic tissues and biofluids, including adipose tissue and blood components [3,4]. During OA, articular cartilage is degraded, synovium becomes inflamed and fibrotic, and components of the synovial fluid bathing the joint, which normally promote smooth joint articulation, is modified to promote disease processes [1]. As the disease progresses, increases in pain and subsequent reductions in physical function and quality of life are common.

Several sociodemographic and anthropometric variables have been defined as risk factors for both the incidence and progression of OA [5,6]. One of the strongest risk factors associated with OA is obesity, which is also the most recognized modifiable risk factor [7]. According to the World Health Organization, individuals with a body mass index (BMI) $\geq$ 30 are considered obese [8], however, this measure does not account for fat distribution or muscle mass. Other measures of obesity that may be relevant include waist circumference, which is a better measure of abdominal fat [9]. Obese individuals put additional mechanical load on their joint tissues, which has been suggested to promote development and progression of OA [10]. However, with changes in systemic body composition, including increased adipose tissue levels, additional molecular modifications are known to occur. For instance, a general increase in systemic inflammation is common in individuals with obesity

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compared to normal weight individuals, with contributions of adipose tissue-associated lipid molecules and cytokines, commonly called adipokines, including leptin, adiponectin and resistin [11–13]. Additional metabolic comorbidities are also associated with obesity, such as type II diabetes [14]. Overall, increased adiposity can modify biomechanics, and local and systemic biofluid compositions, which may ultimately influence joint tissue activity and OA pathology [15].

There has been a recent explosion of studies using various high-throughput omic technologies, including transcriptomics and metabolomics, to investigate molecular entities in various biofluids and tissues associated with OA [16]. Transcriptomics involves the investigation of RNA transcripts in a sample, typically done via probe-based approaches like microarray, or next-generation sequencing. RNA transcripts detected by transcriptomics can include both coding transcripts, such as mRNA, and non-coding transcripts that regulate gene expression through epigenetic mechanisms, such as miRNAs and long non-coding (lnc)RNAs. Metabolomics investigates metabolites in a given biological sample and can be analysed by targeted and untargeted approaches. Untargeted approaches allow for identification of potentially unknown metabolites with relative quantification. In contrast, targeted metabolomics approaches allow for quantification of hundreds of metabolites at once, but do not identify novel metabolites. The metabolome is a direct reflection of the overall metabolic activity (related to gene and protein expression profiles) of a given cell, tissue, organ system, or organism.

In this narrative review, we will explore the contributions of obesity to OA. Specifically, we will focus on studies using transcriptomics and metabolomics from tissues and biofluids from both local and systemic sources to identify molecular differences in obese versus normal individuals and comment on how these differences may modify OA pathology and therapeutic outcomes. Finally, we will discuss the challenges and limitations of studying the transcriptomics and metabolomics of obesity-related OA. Using PubMed, we utilized the search string "(metabolomics OR transcriptomics OR transcriptome OR microRNA OR lncRNA OR RNA sequencing OR microarray) AND (BMI OR obesity OR metabolic syndrome) AND osteoarthritis NOT review" and identified 102 published articles up to Feb 13, 2024. Furthermore, we searched the NCBI GEO database for "osteoarthritis AND obesity" and identified 5 datasets. Articles were specifically evaluated for the use of omic technologies (microarray, sequencing or metabolomics) to investigate links between OA and obesity in patient populations or animal models, with a total of 15 articles of relevance identified (summarized in Table 1), suggesting that additional studies are needed to directly evaluate transcript and metabolite changes associated with obesity in osteoarthritis using omic technologies. Datasets related to described publications are referenced to their accession numbers in the GEO database.

2. Transcriptomics of obesity and OA

Few studies have investigated the contribution of obesity to the transcriptomes of tissues and biofluids of OA patients. Of the 102 publications and 5 datasets identified for screening, 6 unique studies were specifically involved in investigations using transcriptomic technology studying obesity in osteoarthritis. In a study focused on fibroblasts isolated from OA synovium, total RNA sequencing of cultured synovial fibroblasts isolated from synovium of obese and non-obese patients with hip OA (n = 4/group), a total of 615 differentially expressed genes (DEGs) were identified (377 up-regulated, 238 down-regulated), with upregulated genes enriched for pathways linked to inflammatory processes [17]. In addition, 19 lncRNAs were found to be differentially expressed in the fibroblasts based on donor BMI, with MALAT1 increased in obese BMI fibroblasts, responsive to cytokine stimulation, and linked functionally to proliferation of synovial fibroblasts from obese patients.

Five additional studies have focused on transcriptomics of joint tissues from obese OA patients or animal models. In a pre-clinical study investigating the contribution of diet-induced obesity to gut microbiota, inflammation and OA, high-fat-diet fed mice had a loss of beneficial *Bifidobacteria* in the gut, leading to inflammatory intestinal changes identified by RNA-sequencing associated with increased serum and synovial inflammation, and accelerated surgically-induced OA pathology. Interestingly, supplementation with a diet containing oligofructose reversed the changes to gut microbiota, inflammation and OA pathology (GSE98287) [18], supporting associations of diet and the gut to OA pathology in obesity. In a microarray study comparing cartilage from obese (n = 20) versus overweight (n = 3) BMI advanced knee OA patients, a greater number of DEGs were identified in medial (381) vs lateral (112) compartment cartilage, with the majority upregulated in both set-lists (GSE98460) [19]. However, the authors reported only 4 DEGs were shared between the medial and lateral DEG-set lists, suggesting that differential loading on a joint as a result of obesity may have specific effects on chondrocyte gene expression. Up and down-regulated DEGs in medial and lateral cartilage from obese patients were enriched for pathways linked to inflammation, infection, and neurobiology. In a more comprehensive study of OA synovial fibroblasts from hand, hip, knee or foot joints, molecular endotypes of normal versus obese BMI patients were investigated by total RNA and single cell RNA sequencing (n = 24). In total, 416 DEGs were identified as differentially regulated between synovium from normal and obese OA patients with a number of metabolic pathways enriched for the DEG-set list. However, joint site-heterogeneity was also found associated with obese compared to normal BMI patients, with hand, hip, knee and foot synovium having 513, 616, 692 and 672 DEGs respectively, with each DEG-set list having unique pathway enrichment. Finally, the authors found that fibroblasts from hip OA synovium of normal and obese patients organize into distinct clusters based on single-cell sequencing-derived transcriptomic profiles, with obese subsets linked to immune recruitment and fibroblast activation (GSE219027, GSE152815) [20]. In a separate study of synovium of OA patients versus traumatic injury patients, obese BMI was associated with microarray-identified upregulated expression of genes associated with pathways involving protein ubiquitination, endocytosis, endoplasmic reticulum stress and unfolded protein response [21]. Finally, RNA sequencing of infrapatellar fat pad from normal (18.5–24.5 kg/m^2) and obese BMI (>27 kg/m2) Taiwanese patients, as defined by the Taiwanese Ministry of Health, showed 122 genes were significantly differentially expressed in the tissue based on BMI, and were enriched for immune and cell activities such as death, movement, and development [22]. Overall, these studies suggest that joint tissue and cell gene expression profiles are indeed modified by obesity, in part by systemic contributions, and likely impact OA pathology.

Together, these studies indicate that individual joint tissues, joint sites, cells and microbiomes are likely modified by obesity and suggest that obesity is linked to joint tissue and cellular inflammation and functional profiles. However, with the minimal amount of omic data related to site-specific joint tissues, individual cellular constituents, and microbiomes, it is of utmost importance to expand our understanding of these transcriptomes related to obese and non-obese patients to fully understand their differences for precision medicine approaches to OA therapy. In particular, studies focusing on additional biofluids and tissues, other than cartilage, synovium and fecal samples, will be important to appreciate the effects of obesity on OA as both a total joint and systemic disease.

3. Metabolomics of obesity and OA

In contrast to transcriptomic studies of obesity in OA, there have been more metabolomic studies directed to the study of metabolites in obese OA biological samples. Of the 102 PubMed articles identified for screening, 9 specifically used metabolomics to study the effects of obesity on OA. Multiple biofluids have been evaluated for differences in metabolites using metabolomic technologies, including circulating blood. For instance, a comparison between serum from obese (n = 14) and non-obese knee OA (n = 28) patients using untargeted liquid chromatography (LC)-mass spectrometry (MS)/MS metabolomics found different levels of 15 metabolites between the two groups [23]. The metabolites identified

Table 1
Summary of 15 studies identified investigating obesity as a variable related to human or animal model osteoarthritis using transcriptomics or metabolomics. Publications with associated Gene Expression Omnibus dataset accession numbers (GSE) are indicated.

Publication	Human OA/ Animal Model of OA	Comparison Groups Description	Sex	Omic	Technology	Tissue/Cells/Fluid
Nanus et al. (2020) [17]	Human OA	• Obese hip OA • normal-weight hip OA • normal weight non-OA	Male and female	Transcriptomics	Bulk RNA sequencing	Hip OA synovial fibroblasts
Schott et al. (2018) [18] (GSE98287)	Mouse diet-induced obesity	• Low-fat diet-fed mice • high-fat diet-fed mice	Not Reported	Transcriptomics	Bulk RNA sequencing	Colon
Rai et al. (2020) [19] (GSE98460)	Human OA	• Obese knee OA (BMI >30 kg/m2) • Overweight knee OA (BMI <30 kg/m2)	Male and female	Transcriptomics	Microarray	Knee articular cartilage
Wijesinghe et al. (2023) [20] (GSE219027 & GSE152815)	Human OA	• Obese BMI OA (>30 kg/m2) • Normal BMI OA (18.5–24.9 kg/m2)	Male and female	Transcriptomics	Total RNA and single cell RNA sequencing	Hand, hip, knee or foot OA synovial fibroblasts
Roebuck et al. (2022) [21]	Human OA	• Obese BMI end-stage knee OA (>30 kg/m2) • Non-obese BMI end-stage knee OA (<30 kg/m2) • Obese BMI arthroscopy knee OA (>30 kg/m2) • Non-obese BMI arthroscopy knee OA (<30 kg/m2) • Obese trauma non-OA (>30 kg/m2); • Non-obese trauma non-OA (<30 kg/m2)	Male and female	Transcriptomics	Microarray	Knee synovium
Sun et al. (2023) [22]	Human OA	• Normal BMI knee OA (18.5 to less than 24 kg/m2) • Obese BMI knee OA (≥27 kg/m2) (BMI categories based on Ministry of Health and Welfare of Taiwan)	Male and female	Transcriptomics	Bulk RNA sequencing	IFP; IFP-stromal cells; IFP-dedifferentiated fat cells
Senol et al. (2019) [23]	Human OA	• Obese BMI knee OA (≥30 kg/m2) • Non-obese BMI knee OA (<30 kg/m2)	Male and female	Metabolomics	Untargeted LC-MS/MS	Serum
Werdyani et al. (2021) [24]	Human OA	• Primary hip or knee OA • OA-free controls	Male and female	Metabolomics	Targeted LC-MS/MS	Plasma
Zhang et al. (2014) [25]	Human OA	• Primary hip or knee OA	Male and female	Metabolomics	Targeted LC-MS/MS	Synovial fluid
Farah et al. (2022) [26]	Human OA	• Normal BMI hip OA (18–25 kg/m2) • Obese BMI hip OA (>30 kg/m2)	Male and female	Metabolomics	Untargeted ^{1}H NMR spectroscopy	Synovial fluid
Loeser et al. (2016) [27]	Human OA	• Overweight to obese BMI Knee OA (27 to less than 40.5 kg/m2) who were progressors (decrease joint space with of ≥0.7 mm) • Overweight to obese BMI Knee OA (27 to less than 40.5 kg/m2) who were non-progressors (decrease joint space with of ≤0.35 mm)	Male and female	Metabolomics	Untargeted ^{1}H NMR spectroscopy	Urine
Wu et al. (2017) [28] [Also see Wu et al. (2015) [29]]	Mouse diet-induced obesity	• Control low-fat diet-fed mice; • Saturated fatty acid high-fat diet-fed mice; • Omega-6 polyunsaturated fatty acid high-fat diet-fed mice; • Omega-3 polyunsaturated fatty acid high-fat diet-fed mice	Male	Metabolomics	Targeted gas chromatography	Serum and synovial fluid
Meessen et al. (2020) [30]	Human OA	• Knee or Hip OA Cases [subgroup analyses for Prevalence, Total Joint Arthroplasty (BMI <30 or ≥ 30), and Progression]; • Non-OA Controls	Male and female	Metabolomics	Untargeted ^{1}H NMR spectroscopy	Plasma
Hahn et al. (2021) [31]	Mouse diet-induced obesity	• Control 10% fat diet • Control 10% fat diet with exercise • High 60% fat diet • High 60% fat diet with exercise	Male	Metabolomics	Untargeted LC-MS/MS	Synovial fluid
Rushing et al. (2022) [32]	Human OA	• Both radiographic hand OA (3 joints across both hands) and knee OA (KL 2–4 or TKA) with BMI ≥30 kg/m2 • No radiographic hand OA or knee OA (KL 0–1) with BMI ≥30 kg/m2	Male and female	Metabolomics	Untargeted LC-MS/MS	Fecal Samples

BMI, body mass index; KL, Kellgren-Lawrence grade; LC/MS-MS, liquid chromatography/mass-spectrometry-mass spectrometry; NMR, nuclear magnetic resonance; OA, osteoarthritis.

were involved in glycerophospholipid and glycerolipid metabolism, fatty acid biosynthesis, nitrogen metabolism, Aminoacyl-tRNA biosynthesis, glutathione metabolism, vitamin B6 metabolism, butanoate metabolism, and arginine and proline metabolism. In a cohort of 615 primary hip or knee OA patients and 237 controls, targeted metabolomics LC-MS/MS of subject fasting plasma identified 3 clusters of OA subjects. One cluster of OA subjects had higher mean BMI and proportion of diabetics compared with the other two clusters. Concentrations of C4 and PCae (40:3), and their ratio, contributed most to endotype classification, suggesting that these metabolites may be linked to obesity and diabetes in OA patients [24]. In a targeted LC-MS/MS metabolomics study of synovial fluid from 80 subjects from the same OA cohort, two main clusters (A and B) of OA

patients were identified, differentiated primarily by acylcarnitines, but independent of BMI [25]. Interestingly, cluster B could be subdivided into two separate subclusters, with the subclusters differentiated by 86 metabolites, defined mostly by glycerophospholipids, sphingolipids and amino acids, but also with significant differences in BMI between the subgroups. These data suggest that metabolic profiles in biofluids of OA patients are likely related to BMI and metabolic syndromes, like diabetes, and may be important when investigating response to therapy.

Additional studies using synovial fluid or urine have also been evaluated in obese OA populations. Using ^{1}H nuclear magnetic resonance (NMR) spectrometry, synovial fluid of obese (n = 5) and normal (n = 6) BMI OA patients was evaluated to identify differences in the metabolite profiles between the patient groups [26]. Overall, 8 metabolites of 133 detected were significantly increased in synovial fluid of obese BMI patients, including 1,3-dimethylurate, glucose, glycine, lactate, *N*-nitrosodimethylamine, pyruvate, succinate and tyrosine, of which glycine, lactate, succinate, and tyrosine were positively correlated with BMI and waist-to-hip ratio. Furthermore, under inflammatory cytokine stimulation, synovial fibroblasts from obese OA patients had higher lactate secretion, suggesting that cellular metabolism in the joint likely contributes to the metabolome of synovial fluid in an obesity-related fashion. Furthermore, in a sample of overweight and obese adults with OA (n = 44), ^{1}H NMR untargeted metabolomics revealed baseline and change in urine metabolite profiles over 18 months were associated with age, sex and BMI-matched progressors versus non-progressors [27]. Annotated metabolites in signatures that differentiated progressors from non-progressors were enriched for pathways involving amino acid metabolism, lipid metabolism, glycosphingolipid metabolism, and GalNAcbeta1-3Gal. This study suggests disease progression of OA patients with higher BMI may be impacted by additional environmental and sociodemographic factors other than BMI alone, which may include diet.

Pre-clinical studies using metabolomics have investigated the contribution of diet to obesity-related OA progression. In a pre-clinical study of surgically-induced OA, male mice were fed either a low-fat or one of three high-fat diets rich in saturated fatty acids (SFAs), omega-6 or omega-3 polyunsaturated FAs (PUFAs) with the omega-3 PUFA diet influencing OA mouse weight and OA severity [28,29]. Interestingly, diet, but not weight, was found to be significantly associated with OA severity [29]. Further studies using targeted lipidomic analysis of serum from the mice found that omega-3 high-fat diet-fed mice exhibited higher levels of serum omega-3 fatty acids which were generally negatively correlated with OA severity while most omega-6 PUFAs exhibited positive correlations with OA [28]. In synovial fluid of these mice, absolute concentrations of 11/12 fatty acid metabolites were negatively correlated with OA severity, including all reported omega-6 PUFAs [28]. In a separate study, chain length of fatty acids in plasma identified by ^{1}H NMR were also found to be associated with risk of OA or total joint replacement, independent of BMI, in a cross-sectional study of OA patients (n = 1556) compared to controls (n = 2125) [30]. Thus, specific fatty acid metabolite content and acid chain length in diet likely contributes to OA progression in pre-clinical models, however equivalent studies in humans using metabolomics are needed to comprehensively determine how fatty acids in diet contribute to the metabolome of obese OA patients.

The effect of diet and exercise on metabolomes of biofluids has also been evaluated in pre-clinical studies of obesity-related OA. In a study investigating the effects of diet and exercise on OA in adult mice, male mice were fed control or high-fat (60% kcal fat) from age 6–52 weeks, with half the animals given access to a running wheel for exercise from 26 to 52 weeks of age (n = 9–13 per group) [31]. High-fat diet-fed mice had increased body mass and body fat %, and developed moderate OA, as compared to control-fed mice. Access to exercise had very little effect on these parameters or on cartilage damage. However, high-fat diet and exercise had a profound effect on the synovial fluid metabolome, as measured by untargeted LC-MS/MS, with various amino acids, lipids, and steroids modified in distinct diet/exercise groups and enriched to specific of metabolic pathways. Interestingly, correlation-based network analysis determined that exercise may help to re-establish a metabolic link to joint structure, as compared to inflammation in HFD-fed mice alone. Thus, diet and exercise together may be necessary for metabolic and disease modification, but additional pre-clinical and clinical studies are necessary to fully elucidate this possibility.

Finally, fecal samples from obese OA patients have also been evaluated by metabolomics. Untargeted LC-MS/MS metabolomics of fecal samples from obese radiographic OA patients (n = 59), compared to obese individuals with little to no radiographic signs of OA (n = 33) found that the top significant differences in annotated peaks between OA and controls were primarily di- and tri-peptides, with higher levels found in OA patient fecal samples [32]. Leukotriene and tryptophan metabolism were the most enriched pathways linked to differences in OA versus control groups. It would be of interest to evaluate how normal BMI OA patient compares to obese subject fecal metabolomes to define a fecal metabolic signature distinct in obese OA patients and whether a fecal metabolic signature exists for accelerated OA progression in obese OA patients.

Similar to what transcriptomic studies have discovered about relationships between RNA species and obesity-related OA, metabolomics has also revealed modifications to metabolomes of biological samples. Although more studies were identified that have used metabolomics to study metabolism, the number of studies is insufficient to fully appreciate the contributions of obesity to metabolomes of biofluids, and does not begin to evaluate how metabolomes of various joint tissues may be modified by obesity in OA patients. In addition, the identified metabolomics studies are only beginning to understand the links between diet, exercise and obesity-related OA, studies of OA patients necessary to translate pre-clinical insights.

4. Challenges

There are a variety of challenges related to studies investigating links between transcriptomics, metabolomics and obesity in OA (summarized in Figure 1 and Table 2). One of the first challenges that exists is heterogeneity in OA patients. Individuals with OA come from a variety of ethnic backgrounds, and thus individual genetics may influence OA pathology and affect gene expression and ultimately metabolomes of tissues and fluids [33–37]. Patient age and sex are also directly linked to differences in biofluid and tissue metabolomes and transcriptomes [38–43], which may be compounded by obesity. Considering that females, as compared to males, generally have increased adiposity [44], overall body fat may also contribute to females having a higher risk of OA incidence and prevalence, regardless of BMI, compared to males [45], who also report higher pain and lower physical function [46,47]. Thus, considerations for sex and ethnicity would be vital to understand the effect of obesity on OA populations.

Socioeconomics and geographical location can also directly influence local and systemic omic measures, particularly through differences in access to, and affordability of, healthy dietary products [48,49]. In addition, dietary choices may also influence microbiomes, transcriptomes or metabolomes, either autonomously (by the individual) or non-autonomously (from outside sources). Furthermore, ingestion of various dietary fatty acids can have an effect on miRNA expression [50], indicating a link between metabolites and miRNA expression. In addition, xeno-miRNA transfer may also directly modify the transcriptome. Circulating bovine miRNAs have been found in individuals ingesting bovine milk, which could ultimately modify endogenous gene expression and pathways [51,52]. Individuals who rely more heavily on a plant-based diet may also be subjected to plant-based miRNA delivery and associated modification of gene expression, particularly involving

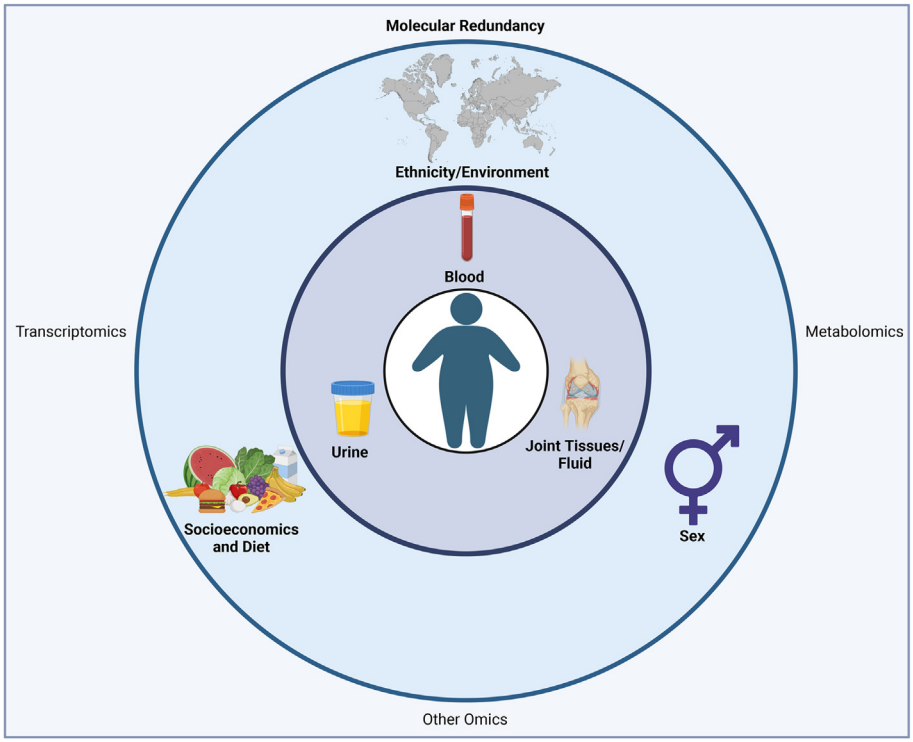

Fig. 1. Challenges using transcriptomics and metabolomics to investigate obesity-related osteoarthritis (OA). Patients who are obese are already differentially categorized from normal BMI individuals, but additional sociodemographic and biological variables may impact the heterogeneity of transcriptomes and metabolomes that can be detected through omic technologies. This heterogeneity can be influenced by various interactions between joint tissues and local or systemic fluids, in addition to direct or indirect impacts of diet, ethnicity, environment, economic status and sex. Compounding these challenges is the variety of functional redundancy that exists in the transcriptome, metabolome, and other "omes", where specific molecules can be exchanged for one another, resulting in similar effects. Created with BioRender.com.

nervous system pathways [53]. Diet has also been shown to impact metabolomes. For instance, a 12-week low-carbohydrate and high fat diet modified plasma lipid profiles, regardless if high-interval training exercise was included [54], while adherence to a Mediterranean diet can elicit a specific lipid metabolic profile [55]. In pre-clinical studies, a variety of diets were shown to impact the metabolomes of mice based on sex [56]. A completed clinical trial also investigated how prebiotics influence metabolomes, inflammation, pain and joint function in obese patients with OA (NCT04172688) [57], however, results have not been provided to date. As detailed diet information is typically not acquired prior to OA biofluid or tissue collection, our understanding of OA patient metabolomes and their associated risk of progression or therapeutic outcomes becomes more complicated. Overall, sociodemographic, environmental and anthropometric variables not only influence OA pathology, but can have profound effects on metabolomes and transcriptomes obese individuals. Thus, larger OA cohorts that encompass the diversity of BMI, ethnicity, sex, and diet in OA affected patients may be necessary to fully unravel the contributions of obesity to OA pathology by evaluating models for associations to these important variables. As costs associated with using omic technologies may become limiting as larger cohorts are generated, the use of well annotated transcriptomic and metabolomic datasets deposited in appropriate repositories can also be considered to increase power of detecting smaller signals in the data [58], however caution should be taken when integrating such datasets due to potential sources of variation, such as technology used and experimental design [59].

Joint biology alone can also play a significant role in obesity-related OA. As described herein, individual joint tissues and biofluids have been investigated with respect to metabolomic and transcriptomic changes associated with obesity in OA; however, interactions of joint tissues via paracrine mechanisms may also influence how tissues react and respond during OA to obesity [13,60]. The same can be true of how joint and systemic tissues may interact via miRNA and metabolite transfer between target tissues [61–65]. Thus, understanding the interactions between transcriptomes and metabolomes of local joint and systemic tissues and fluids will help to understand how obesity modifies OA and identify better therapeutics for modifying OA disease in obese individuals. Of note, metabolic activity may be investigated using stable isotope analysis studies, where isotope-labelled (e.g. deuterium or C^{13}) metabolites and subsequent metabolic products can be traced by MS or NMR and define specific metabolic networks modified by obesity in OA, a process known as metabolic flux analysis [66]. Contributions of metabolites from

Table 2
Summary of challenges in obesity-related OA studies, and descriptions of potential contributing factors to consider when studying obesity-related OA using transcriptomics and metabolomics.

Challenge	Influence on obesity-related OA metabolomic and transcriptomic studies
Sociodemographics	• Adiposity is greater in females and may influence metabolomes and transcriptomes • Age and sex directly impact metabolomes and transcriptomes
Genetics	• Ethnic background and environment can directly impact transcript expression
Diet	• Food choices based on geographical location can influence microbiome, metabolomes and transcriptomes • Metabolites from diet can influence miRNA expression • Xeno-miRNA transfer from diet • Diet effects on the metabolome may be sex-dependent
Tissue/fluid interactions	• Paracrine tissue interactions in the local joint space may influence joint biology related to obesity • Sharing of metabolites and transcripts between local joint tissues and the systemic circulating may influence OA progression related to obesity
Molecule redundancy	• Multiple metabolite isotypes may (or may not) be common ligands for the same enzyme • Many miRNAs share common transcript targets

miRNA, microRNA; OA, osteoarthritis.

individual local joint tissues could then be linked to presence in systemic fluid, or vice-versa, depending on metabolite isotope delivery location.

MiRNAs and metabolites also have a vast amount of redundancy with respect to their targets. There is considerable redundancy in our genome, where multiple genes have the same, or similar biological functions [67]. There is also considerable overlap with miRNAs and their transcript targets [68]. Similarly, a variety of metabolites, can be interchanged as ligands for specific metabolic enzymes [69]. As a result, heterogeneity of transcriptomic and metabolite differences across obese individuals with OA are possible, but may result in the similar pathological outcomes. Therefore, single miRNA or metabolite level differences associated with obesity-related OA may be more difficult to identify, and targeting a single miRNA or metabolite may only produce partial therapeutic responses due to this redundancy. To overcome this challenge, the use computational and machine learning techniques to investigate networks of miRNA [70] and metabolite [71] signatures highly associated with obesity-related OA may be better to discover targets to monitor or treat obese OA patients.

5. Conclusions

Of the few studies investigating the transcriptomic and metabolomic links between obesity and OA to date, it is clear that obesity modifies both the metabolomes and transcriptomes of biological tissues, fluids and material, with likely contributions to OA incidence and progression. However, additional efforts are needed to fully elucidate the contributions of obesity to transcriptomic and metabolomic changes in individual tissues and biofluids. As part of future studies, considerations for socio-demographic and anthropometric variables will be necessary to comprehensively understand the impact of obesity on OA. Furthermore, additional focus on transcriptome and metabolome redundancy, and signaling crosstalk between tissues and biofluid, will help to uncover therapeutic targets more selective for obese individuals with OA.

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Contributions

JR, PP, and MK were involved in the conception and design of the manuscript, drafting and critical review of intellectual content. JR, PP, and MK approved the final version to be published and are jointly accountable for all aspects of the manuscript. JR, PP and MK will ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of competing interest

The authors declare no conflicts of interest.

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