Review

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Bioanalysis

Application and prospect of metabolomics in the early diagnosis of osteoporosis: a narrative review

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This paper reviews the application of metabolomics in the early diagnosis of osteoporosis in recent years. The authors searched electronic databases for the keywords 'metabolomics', 'osteoporosis' and 'biomarkers', then analyzed the relationship between functional markers and osteoporosis using categorical summarization. Lipid metabolism, amino acid metabolism and energy metabolism are closely related to osteoporosis development and can become early diagnostic markers of the condition. However, the existing studies in metabolomics suffer from varying application methods, difficulty in identifying isomers, small study cohorts and insufficient research on metabolic mechanisms. Consequently, it is important for future research to focus on broadening and standardizing the scope of the application of metabolomics. High-quality studies on a large scale should also be conducted while promoting the early diagnosis of osteoporosis in a more precise, comprehensive and sensitive manner.

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Osteoporosis is a systemic metabolic bone disease characterized by reduced bone mass and microarchitectural degeneration of bone tissue [1]. It occurs in the elderly and postmenopausal women and has become a severe public health problem in the increasingly aging population [2]. The sensitivity of biochemical indicators for early clinical diagnosis of osteoporosis is currently low, and there is an urgent need to identify novel potential biomarkers to predict the occurrence of bone loss. The pathogenesis of osteoporosis has recently been found to be related to metabolites produced by cellular and tissue activities. Various metabolites such as lipids, amino acids, carbohydrates, organic acids and vitamins are involved in various metabolic pathways, interacting with the endocrine and nervous systems to form a complex network [3].

Factors such as genetic modifications, environmental stimuli and pathophysiological states influence metabolic reprogramming in the body. Metabolomics, as a high-throughput emerging technology, can qualitatively and (semi-)quantitatively analyze endogenous small molecule metabolites ($\leq$1000 Da) and reflect metabolic changes in different states of the body by constructing dynamically changing multimetric metabolic profiles [4,5]. Specifically, the metabolomic research process is divided into three stages: sample preparation, metabolite analysis and data analysis. First, suitable biological samples such as blood, urine, tissues and cells are collected and prepared [6]. Second, metabolites are preprocessed using LC–MS, GC–MS and NMR analysis platforms to collect and analyze

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the data and obtain essential metabolite characteristics [4]. Finally, with the help of multivariate statistical analysis to screen relevant substances and search for information on metabolites, proteins and genes in the network database, researchers can confirm the structure, quantify the target metabolic pathways and analyze the complex biological nature [7,8]. Metabolites are downstream molecules of quantitative bioinformatics, and subtle changes in gene transcription and protein translation, which are difficult to detect in other omics, can be enhanced at the level of metabolomics, making it closest to the pathophysiological phenotypes of organisms [9]. The study of metabolomics in osteoporosis is beneficial for clarifying etiology, diagnosing a disease and providing early intervention.

Therefore, to provide a theoretical reference for exploring the early potential biomarkers and pathogenesis of osteoporosis, the authors searched electronic databases such as PubMed, Web of Science and Google Scholar using the keywords "metabolomics", "osteoporosis" and "biomarkers". They included literature related to the development of osteoporosis and excluded literature on metabolomic changes due to the application of pharmacological interventions. Characteristically altered metabolites from the included literature were categorized and summarized. This article describes the application of metabolomics in the early diagnosis and prediction of osteoporosis from three aspects: lipid metabolism, amino acid metabolism and energy metabolism. Additionally, it discusses the current progress in exploring osteoporosis metabolites based on different metabolomic techniques, such as MS and NMR. Special emphasis is given to comparing existing technologies for application in osteoporosis.

Lipid metabolism in osteoporosis

Fatty acid metabolism

In recent years, various studies have demonstrated a close association between lipid and bone metabolism [10–12]. Fatty acids (FAs) and their metabolites are essential for bone health, especially n-6 FAs and n-3 FAs; n-6 FAs can inhibit osteoblast differentiation by upregulating PPARγ and lipogenesis, while n-3 FAs stimulate osteoblast proliferation by downregulating PPARγ [13]. Clinical studies by Aleidi [14] and Kou [15] revealed abnormal serum unsaturated FA metabolism in postmenopausal osteoporosis (PMOP) patients. Not coincidentally, Yu *et al.* [16] observed the same results in urine samples from menopausal patients. Ma *et al.* [17] used nontargeted GC–MS to detect plasma from ovariectomized rats and found a significant increase in the expression of arachidonic and linoleic acids in n-6 FAs and a significant decrease in docosahexaenoic acid levels in n-3 FAs. This suggests that the occurrence of osteoporosis may be associated with an elevated n-6/n-3 FA ratio. Li *et al.* [18] did not use blood as the test sample and instead analyzed bone tissue metabolic profiles. They discovered a causal relationship between reduced bone mass and a significant decrease in α-linolenic acid in n-3 FAs in osteoporosis model rats. Meanwhile, Sharma *et al.* [19] and Chen *et al.* [20] demonstrated that n-3 FAs could hinder osteoclast differentiation by inhibiting OPG/RANKL and NF-κB-iNOS-COX2 signaling pathways. The latter noted that the metabolic disorders of osteoporosis-related FAs involved palmitic acid, stearic acid and arachidonic acid, among which the reduction of the arachidonic acid level was more significant.

Phospholipid metabolism

Sphingolipids and glycerophospholipids are two classes of phosphate-containing complex lipids. Hydrolysis of glycerophospholipids produces phosphatidylcholine (PC) or is linked to bases to form phosphatidylethanolamine, which is involved in various metabolisms and signal transduction, such as nerve impulses in the body [21]. Additionally, Deng *et al.* [22] targeted the analysis of lipid-related metabolites in serum. In their study, it was identified that patients with osteoporosis primarily exhibited glycerophospholipid, sphingolipid, FA and bile acid metabolism disorders, while PC and phosphatidylethanolamine were upregulated most significantly in glycerophospholipids. Notably, Fang *et al.* [23] observed the opposite result to Deng's [22] study. They found that plasma glycerophospholipid levels in ovariectomized rats did not increase but exhibited a decreasing trend. This contradictory result was investigated by Mei [24] and Zhao [25], who revealed that there are numerous isomers of PC, and the increase or decrease of metabolic levels of different structural isomers was not consistent with osteoporosis. Meanwhile, Mei's [24] study was slightly different in that five metabolites (PC [O-18:0/22:6], sphingolipids, inosine, hypoxanthine, isoleucine-proline) were identified to have an association with higher bone mineral density (BMD) by examining plasma samples from a multicenter source in southeastern China. At the same time, elevated levels of PC (16:0/18:3) were associated with reduced BMC, suggesting that the PC isomers PC (O-18:0/22:6) and PC (16:0/18:3) are positively and negatively correlated with bone mineral content, respectively. Lu *et al.* [26] constructed a cellular model of aged osteoporosis and found that the vast majority of aging bone marrow-derived mesenchymal stem cells in late passages had increased levels of glycerophospholipids, while sphingolipids and a small fraction

of glycerophospholipids decreased, suggesting that differential metabolic changes do exist in glycerophospholipid isoforms. The authors' team hypothesizes that the differences in identifying metabolite isomers are also related to whether the analytical method chosen is targeted metabolomics. Targeted metabolomics, which is mainly used to test exploratory, experimental hypotheses by detecting a preset group of specific metabolites, has the advantages of high specificity and accurate quantification and can better identify isomers. In contrast, untargeted metabolomics aims to measure as many metabolites as possible, which helps in the discovery of new biomarkers and pathways but is not applicable to characterizing subtle differences between metabolites. Therefore, researchers should choose the appropriate platform for targeted/untargeted metabolomics according to the differences in metabolite physicochemical properties, sample nature and experimental objectives.

Solid alcohol metabolism

Solid alcohols are closely associated with the development of osteoporosis. Similarly, bile acids, as end products of cholesterol metabolism, can regulate the bone remodeling process by affecting the absorption of intestinal fat-soluble substances [27,28]. Qiao *et al.* [29] used ultra-high performance LC–MS/MS to analyze stool samples from osteoporosis patients and found that an altered abundance of intestinal flora and increased levels of lithocholic acid resulted in increased calcium and phosphorus absorption and decreased BMD in the organism [30]. As a steroid hormone, androgens have a direct regulatory effect on bone growth and development [31]. Moayyeri *et al.* [32] and Chen *et al.* [20] performed Mendelian randomization analysis on the entire metabolome of Hong Kong and European populations and obtained consistent results. These studies demonstrated that androgen metabolites were directly related to hip and spine BMD. Notably, androstenedione sulfate was found to have a systemic regulatory effect on the maintenance of whole-body bone mass, suggesting the potential early predictive value of androgens in the onset of osteoporosis.

Amino acid metabolism in osteoporosis

Branched-chain amino acid metabolism

Branched-chain amino acids (BCAAs) include valine, leucine and isoleucine, all of which are metabolized in the human body in the same direction and possess the same magnitude of change to regulate bone matrix production [33]. In this regard, Panahi *et al.* [34] found that BCAAs in women and tryptophan levels in men were critical amino acids negatively associated with bone loss in middle-aged and elderly populations, suggesting that different gender populations have gender-specific critical amino acids on which metabolomics can be targeted for analysis and early prediction of osteoporosis onset. Using a Mendelian randomization study, Cui *et al.* [35] revealed a causal relationship between valine and isoleucine in BCAAs and BMD. Meanwhile, it was also found that the occurrence of osteoporosis is closely related to intestinal microecology and renal function. Ling *et al.* [36] discovered that the metabolic levels of BCAAs in osteoporosis patients were more associated with intestinal flora homeostasis. For example, low serum valine levels were significantly and negatively correlated with the increased incidence of osteoporosis, and this result is consistent with two osteoporosis-related metabolomic studies by Palacios [37,38]. In another instance, Li *et al.* [18] used GC–MS to analyze kidney and bone tissue samples from ovariectomized mice and found significantly lower levels of BCAAs and the presence of the common metabolite N-methyl alanine in bone and kidney tissue. Such findings suggest that the occurrence of osteoporosis is associated with the metabolic levels of BCAAs and kidney function.

Aromatic amino acid metabolism

Amino acids with a benzene ring in their molecular structure are known as aromatic amino acids, which include phenylalanine, tyrosine and tryptophan. Phenylalanine and tryptophan are essential amino acids that must be obtained through dietary sources. In contrast, phenylalanine can be converted to tyrosine through hydroxylation in the liver and kidneys and is structurally similar. Aromatic amino acids activate anabolic pathways related to bone remodeling under physiological conditions and positively affect the maintenance of bone mass in the body [39]. By constructing a testicular removal model in rats, Ge *et al.* [40] identified seven significantly different metabolites in the skeletal muscle metabolic profile, including phenylalanine, tyrosine and tryptophan. This suggests that the occurrence of osteoporosis in older men is associated with abnormal metabolism of aromatic amino acids. Additionally, aging causes tryptophan to be metabolized to kynurenine [41], and excess kynurenine inhibits bone formation [42]. The above findings illustrate the existence of characteristic biomarkers in different types of osteoporosis patients. For example, tryptophan levels rise in elderly osteoporosis patients, leading to a decrease in

bone mass. Both Kim [43] and Apalset [44] found significantly higher levels of kynurenine in people with declining hip bone mass, and the latter also revealed that the kynurenine/tryptophan ratio was negatively correlated with hip BMD. In another study, Ling *et al.* [36] used targeted metabolomics to analyze stool samples from osteoporosis patients. Here, it was found that both lumbar tyrosine levels and femoral neck tryptophan levels were high, with tryptophan metabolism levels differed from Apalset's [44] results, presumably due to differences in metabolites for bone loss at different sites. They are also related to differences in diverse sample sources. Notably, future studies must explore the relationship between different sites, sample biomarkers and osteoporosis pathogenesis, especially the association between different osteoporosis-prevalent sites and their characteristic metabolites, such as the vertebrae, distal forearm, hip, proximal humerus, pelvis and femoral neck.

Other amino acid metabolism

Glycine is a common metabolite associated with BMD levels. Miyamoto *et al.* [45] found that serum glycine was considerably elevated in patients with osteoporosis. Meanwhile, Zhang *et al.* [46] analyzed plasma samples with the aid of LC–MS/MS and found that higher levels of glycine were associated with decreased bone mass in the femoral neck and lumbar spine. These findings indicated that blood glycine levels were positively associated with the occurrence of osteoporosis. In addition, Pernow *et al.* [47] and Eriksson *et al.* [48] demonstrated that femoral neck fractures were associated with circulating levels of glycine in Swedish men, and the former also found that plasma nonessential amino acids such as glycine were negatively correlated with BMD in male patients with idiopathic osteoporosis, whereas essential amino acid levels were normal. However, both Jennings *et al.* [49] and Kim's [50] study discovered that oral glycine in women had a protective effect on bone. Li *et al.* [51] hypothesized that, due to its high affinity for estrogen receptor α, glycine could stimulate osteogenic differentiation via estrogen receptor-related signaling pathways in women, and future studies should focus on analyzing the underlying reasons for this contradiction. Ma *et al.* [17] revealed a significant increase in plasma hydroxyproline levels in ovariectomized rats, and hydroxyproline levels increased during bone matrix depletion, indicating a negative correlation between hydroxyproline and BMD. In another study, Wang *et al.* [52] employed MS to analyze serum samples from osteoporosis patients. Here, it was found that arginine, glutamine, histidine and serine in men and glycine and t4-OH-Pro in postmenopausal women were associated with BMD. Among these, glutamine can regulate bone metabolism via osteoclasts and can be converted to glutamate, leading to bone resorption via glutamate receptor expression on osteoblasts. This further suggests that metabolomics can predict the development of osteoporosis in men and women based on the detection of different amino acids.

Energy metabolism in osteoporosis

Carbohydrate metabolism

Adenosine triphosphate is a direct energy source for bone remodeling, and carbohydrates such as glucose and galactose are the preferred substrates for adenosine triphosphate production by pathways such as glycolysis and oxidative phosphorylation. Cipriani *et al.* [53] found that bone metabolism is associated with endocrine metabolisms, such as glucose homeostasis, insulin sensitivity and energy metabolism. In humans, the effect of glucose metabolism on osteoblasts suggests a crosstalk between bone and glucose homeostasis, a critical mechanism predisposing patients with Type 1 and Type 2 diabetes to osteoporosis. High glucose and hypoxia are characteristic pathological microenvironments of diabetic osteoporosis. Zhang *et al.* [54] and Wang *et al.* [55] demonstrated that the HDAC4/HIF-1α/VEGFA axis plays a vital role in developing diabetic osteoporosis. Histone deacetylases, as transcriptional coblockers, regulate the activity of HIF-1α, which plays a dominant role in the hypoxic environment and establishes a link with high glucose. HIF-1α regulates mitochondrial function through the hypoxia–high glucose axis, maintaining energy metabolism homeostasis and activating VEGFA to generate reactive oxygen species in response to cellular hypoxia.

Apart from diabetic osteoporosis, abnormal carbohydrate metabolism is observed in other types of osteoporosis. A study conducted by Kang *et al.* [56] illustrated an increase in glucose and lactate levels and a decrease in energy expenditure by observing the metabolic levels of glucose and lactate in ovariectomized rats. This suggests that impaired utilization of substrates such as glucose may lead to an imbalance in energy metabolism, which induces the development of osteoporosis. Yu *et al.* [16] analyzed urine samples from PMOP patients using MS. This study observed a significant increase in galactose levels along with a degree of insulin resistance in patients. Additionally, it was found that galactose directly inhibits follicular development and leads to estrogen reduction and bone loss, tentatively demonstrating the potential of galactose as an early diagnostic indicator of osteoporosis [57]. Wang

et al. [58] used bilateral ovariectomized rats as an animal model, tested their serum samples and screened five differentials as potential biomarkers: glucose, glycine, lysine, tryptophan and docosahexaenoic acid. Among these, three of the metabolites were significantly elevated and tryptophan was significantly decreased. In conclusion, carbohydrate metabolism, especially glucose metabolism, plays an integral role in the development of osteoporosis.

Organic acid metabolism

Taurine is an endogenous antioxidant that promotes osteoblast differentiation and improves the bone metabolism index [59]. Yuan *et al.* [60] revealed that taurine has a direct link to bone homeostasis, which regulates osteoblast metabolism and promotes osteoblast differentiation by the ERK pathway. Meanwhile, Yu *et al.* [16] detected a sustained decrease in taurine levels in the urine of osteoporosis patients, and this process coincided with the onset of osteoporosis. Yu *et al.* [16] also revealed significantly increased levels of β-alanine, succinic acid and 5-hydroxy hexanoic acid. Pyruvate is the end product of glycolysis and is involved in the energy metabolism of osteoblasts [61]. Qi *et al.* [62] employed the GC–MS technique to analyze serum samples from PMOP patients and found that, the lower the pyruvate content, the lower the BMD and the two were positively correlated. Additionally, some organic acids can be released by ecologically imbalanced intestinal microbiota and affect the development of osteoporosis through the 'microbe–gut–bone axis' [63,64]. For example, Greenbaum *et al.* [65] analyzed approximately 80 intestinal metabolites. It was found that propionic acid, butyric acid, valeric acid and uric acid among the organic acids were all associated with BMD levels. This includes propionic acid inhibiting osteoclast-related genes such as *TRAF6* and *NFATC1* [64], butyric acid regulating Wnt10b signaling to promote bone anabolism [66] and uric acid inhibiting bone formation via the intracellular oxidative stress pathway [67]. All of the above organic acid metabolites are produced by mitochondria and participate in energy metabolic processes, indicating that organic acid metabolism has some reference value in the early prediction of osteoporosis.

Challenges of metabolomics in early diagnosis of osteoporosis

As a postgenomics tool that can process small molecule metabolites such as lipids, amino acids, carbohydrates and organic acids within a given time frame, metabolomic technology provides systematic and comprehensive information to reflect the pathological and physiological status of living organisms. This certainly has its unique advantages compared with imaging and laboratory tests. Dual-energy x-ray absorptiometry is the 'gold standard' for diagnosing osteoporosis and can predict the onset of osteoporosis at an early stage. However, frequent examinations can overexpose the body to radiation and increase the risk of cancer [68]. Dual-energy x-ray absorptiometry is susceptible to factors such as human height, scoliosis, osteophytes and degenerative changes, resulting in less accurate bone density measurements [69]. Metabolomics employs techniques such as MS to analyze human blood, urine and stool samples, which are safe and less susceptible to interference with the measurement results, making it more suitable for early prediction of the occurrence of osteoporosis. Meanwhile, alkaline phosphatase, calcium and phosphorus level; 25-hydroxyvitamin D; and biochemical markers of bone turnover are often used as adjunctive diagnostics for osteoporosis. However, their specificity and sensitivity for early disease prediction could be improved [70]. Metabolites are located downstream of systems biology and can amplify subtle gene and protein expression changes. Therefore, metabolomics is closer to the phenotype of an organism than existing laboratory tests and can more directly reflect the organism's function [9].

Metabolomics is an emerging histological technology still in its initial stage of development. This paper reviews existing studies and notes that various aspects still need improvement in applying metabolomics for the early prediction of osteoporosis. First, the lack of standardized methods for metabolomic studies, especially the choice of experimental methods, sample collection and raw data preprocessing methods, can significantly affect the stability of metabolites. This can result in biased metabolic profiles obtained from different studies [71]. For example, blood is a collection of endogenous metabolites that reflect the vital activities of the entire biological system at a specific time. The studies of Kou [15] and Ma [17] used serum and plasma as sample sources to analyze the metabolic characteristics of PMOP, respectively. Kou *et al.* [15] added anticoagulant to serum samples and then centrifuged serum immediately at 16,000 × *g* for 15 minutes, which has the advantage of being immediately placed on ice and avoiding the adverse effects of sample exposure at room temperature. The treated serum was free of coagulation factors and platelets, thus enabling less interference from metabolites produced by the natural clotting process. In contrast, Ma *et al.* [17] collected serum and left it at room temperature to produce serum through natural clotting. The clotting process leads to the release of multiple metabolites from activated platelets, which can interfere with the data statistics and ultimately result in differential results of metabolic profiles. Therefore, to reduce systematic bias in metabolomics

and improve the accuracy of the early prediction of osteoporosis, scholars should establish and improve standardized protocols for metabolite extraction, sample storage and data normalization in metabolomics [72,73].

Second, it is important for metabolomics to address the problem that the sensitivity and coverage of individual platforms need to be higher to comprehensively cover all metabolites in an organism [74]. Additionally, future studies should aim to improve the accuracy of each platform to capture subtle differences among early metabolic phenotypes. Specifically, MS, a widely used technique for endogenous metabolite analysis, can provide qualitative and quantitative information. However, since primary MS obtains the composition of the substance to be measured by detecting the ion-to-mass charge ratio of small molecules, it cannot identify isomers, and MS/MS is equally challenging to distinguish isomers with common cleavage pathways. Therefore, it is crucial to improve the selectivity and application scope of MS for isomers [75]. Significant differences in the identification of isoforms have been identified in the studies of Deng [22], Mei [24], Zhao [25] and Fang [23] in this paper, and the authors speculate that the reasons for these differential results may be the following: 1) the high variability in populations of different races, ethnicities and regions, including linkage disequilibrium and differential expression of genetic phenotypes; 2) differences in metabolomic techniques applied by different subject groups, such as differences in column and mobile phase ratios, and the use of targeted analysis (nontargeted metabolomics reflects the total metabolite profile but has low sensitivity, while targeted metabolomics quantifies the target metabolites and obtains their absolute content and subtle differences; therefore, the combined use of targeted metabolomics is beneficial for the separation and identification of isomers); and 3) different experimental designs, such as sample size, whether it is a multicenter source sample and the type of osteoporosis model being used. Aging, menopause and diabetes-induced osteoporosis may have their own characteristic metabolic profiles.

In addition, current applications of metabolomics in osteoporosis mostly focus on cross-sectional cohort studies [20,29,65,76–79] and small, single-center, exploratory studies [15,22,25,29,65,80]. Cross-sectional studies elucidate only the correlation between metabolites and the occurrence of osteoporosis, making it challenging to determine the causal relationship between the two, which hampers the translation to early clinical diagnosis. Therefore, future studies should use Mendelian randomization analysis to infer the etiology and confirm the findings through longitudinal clinical trials. Furthermore, given the considerable genetic heterogeneity among subjects, large-scale, multicenter clinical studies are needed to address these issues and validate the results obtained from animal experiments in human studies. This will enhance the validity and reliability of the findings and facilitate the development of individualized early diagnostic criteria for different populations.

Conclusion

As osteoporosis is a progressive systemic orthopedic disease, potential biomarkers from chronological and longitudinal metabolomic data at the cellular and tissue levels play a crucial role in bone homeostasis and the development of osteoporosis, which is a popular current research focus. Metabolomics mainly depicts metabolic snapshots with the help of MS or NMR techniques to detect potential osteoporosis-related biomarkers and can identify previously unknown molecules involved in the onset and progression of osteoporosis. Metabolomics studies have been used to search for potential biomarkers for screening, diagnosing, and predicting osteoporosis in animal models and clinical studies. Lipids, amino acids, carbohydrates and organic acids are potential biomarkers identified by existing metabolomics studies as strongly associated with osteoporosis development. Researchers have monitored samples from different sources to identify characteristic key markers and establish associations between potential biomarkers and different types of osteoporosis, low BMD at different skeletal sites and osteoporosis patients of different genders. Monitoring metabolite-related biomarkers and skeletal homeostasis not only facilitates the early diagnosis and prognosis of osteoporosis but also helps prevent the onset and progression of the disease.

Future perspective

Despite the overall maturity and widespread application of metabolomics, it is still an emerging technology that needs improvement in osteoporosis research. Based on the available technology and the diversity of the physicochemical properties of metabolites, no single assay technique can simultaneously identify all metabolites in a biological sample. Therefore, different analytical methods must be used, depending on the disease being studied, the properties of the metabolite and the specific objectives of the study.

Notably, the metabolomics-based studies of osteoporosis reported in the literature have different methods of metabolomic application, focus on a single study target and contain insufficient depth of metabolic mechanism research. The accuracy and depth of these studies still need improvement. Additionally, existing studies almost

exclusively use untargeted metabolomic approaches. Thus, future research could focus on improving the sensitivity and coverage of metabolomics-related technologies and conducting large-scale, multicenter, high-quality longitudinal studies. Meanwhile, combining targeted metabolomic studies with untargeted metabolomics is indispensable to improve selectivity and accuracy. Based on these findings, our team has proposed combining metabolomics with traditional osteoporosis diagnostic methods to improve the sensitivity and accuracy of early disease diagnosis. Additionally, linking metabolomics with other systems biology datasets, such as proteomics, transcriptomics and genetics, will further deepen our understanding of the complexity of osteoporosis. Such methods will help provide personalized medical treatment to patients, ultimately improving clinical prognosis and regression. Altogether, much work remains to be done to validate and confirm potential biomarkers associated with the development of osteoporosis, which will require a significant workforce, technology and often a large number of researchers.

Executive summary

Lipid metabolism associated with osteoporosis

- The metabolic disorders in osteoporosis are dominated by abnormal lipid metabolism, mainly mediated by PPARγ. PPARγ activation inhibits osteoblast differentiation, leading to the disruption of bone homeostasis.
- Fatty acids (FAs) play an essential role in the regulation of bone metabolism, and the development of osteoporosis may be associated with an elevated n-6/n-3 FA ratio. Additionally, disorders of FA metabolism in osteoporosis involve palmitic, stearic and arachidonic acids.
- Phosphatidylcholine and phosphatidylethanolamine are phospholipids more closely related to osteoporosis metabolism, and care should be taken to identify their isomers when isolating glycerophospholipids.
- Bile acids are the end products of cholesterol metabolic degradation and can regulate the bone remodeling process by influencing the intestinal absorption of fat-soluble substances. Therefore, analyzing fecal samples from osteoporosis patients is more conducive to identifying sterols related to osteoporosis development.

Amino acid metabolism associated with osteoporosis

- Development of osteoporosis is linked to the metabolic levels of branched-chain amino acids, aromatic amino acids, glycine and glutamine. These levels can regulate the production of bone matrix through renal interactions.
- Patients with osteoporosis of different genders exhibit specific vital amino acids: branched-chain amino acids, glycine and t4-OH-Pro are prevalent in females, while tryptophan, arginine, glutamine, histidine and serine are prominent in males.
- The metabolite profiles of osteoporosis vary from different skeletal sites: the kynurenine/tryptophan ratio is distinct in the hip joint, tyrosine levels in the lumbar spine and both tryptophan and circulating glycine levels in the femoral neck.

Energy metabolism associated with osteoporosis

- Bone remodeling processes require a substantial amount of energy, including carbohydrate and organic acid metabolism. When energy metabolism is disturbed, it significantly impacts the bone remodeling process.
- The presence of mutual crosstalk between bone and glucose homeostasis is a crucial factor in the development of diabetic osteoporosis.
- Taurine, pyruvate, propionic acid, butyric acid, valeric acid and uric acid are organic acids closely involved in osteoporosis development. They are produced by mitochondria and play a role in energy metabolism. Some of these organic acids also influence osteoporosis development through pathways such as the 'microbial–gut–bone axis.'

Challenges of metabolomics in osteoporosis research

- Metabolomics is still in the initial development stage in osteoporosis research, and standardized methods are lacking in its study. Researchers should establish protocols to standardize the procedures, aiming to reduce systematic differences in metabolic profiles of osteoporosis and improve the sensitivity and accuracy of potential biomarkers.
- There are technical bottlenecks in metabolomic analysis platforms, leading to insufficient sensitivity and coverage. For instance, the low selectivity and limited application range of MS analysis techniques for isomers result in metabolic profiling results that comprehensively cover only some metabolites of an organism.
- The application of metabolomics in osteoporosis mainly focuses on cross-sectional studies. Consequently, it is challenging to determine the causal relationship between osteoporosis occurrence and differential metabolites. Future studies should consider adopting Mendelian randomization analysis to determine the causal relationship and further confirm it through longitudinal studies.
- Most existing studies use nontargeted metabolomic methods, which have the limitations of low specificity and insufficiently accurate quantification. However, targeted metabolomics can detect subtler differences between metabolites by focusing on specific predefined metabolites. Therefore, future research must incorporate both targeted and nontargeted metabolomic approaches to achieve more precise individualized treatment.

Author contributions

Y Li and Y Si reviewed the relevant literature, collected and analyzed the data and wrote the manuscript. Y Ma and H Yin conceived and designed the study, supervised the work and assisted with the writing, review and editing of the manuscript, as well as funding acquisition. All authors have approved the final version of the manuscript.

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