Review

Osteoporosis and gut microbiota, radiofrequency echographic multi-spectrometry and machine learning: one year in review 2023

M. Maffi, G. De Mattia, M. Mazzantini

Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Italy

*Michele Maffi, MD**
*Giammarco De Mattia, MD**
Maurizio Mazzantini, MD, PhD

**These authors contributed equally.*

Please address correspondence to:
Michele Maffi
Reumatologia, Dipartimento di Medicina Clinica e Sperimentale, Università di Pisa.
Via Roma 67,
56126 Pisa, Italy.
E-mail: michele.maffi93@gmail.com

Received on January 7, 2023; accepted in revised form on April 26, 2023.

Clin Exp Rheumatol 2023; 41: 1377-1383.

Key words: osteoporosis, gut microbiota, REMS, machine learning

Competing interests: none declared.

ABSTRACT

Osteoporosis is a prevalent bone disease with a relevant burden of mortality and comorbidity, especially due to fragility fractures occurring as a result of reduced bone mineral density. In this review we provide a critical digest of the most recent literature regarding the relationship between gut microbiota and osteoporosis and discuss the role of radiofrequency echographic multi-spectrometry (REMS) and machine learning in the diagnostic work-up and prevention of osteoporosis.

Introduction

Osteoporosis (OP) is a skeletal disease characterised by low bone mineral density (BMD) and deterioration of bone tissue microarchitecture. It is the most prevalent bone disorder in adults, especially among postmenopausal women, and is frequently associated with fragility fractures, resulting in increased morbidity and mortality, in a lower quality of life, and in a remarkable social and economic burden (1).

The aim of this review was to provide an overview of recent advances in the field of OP. Given the large number of publications, we focused on three main topics: the role of gut microbiota in the pathogenesis of OP, and radiofrequency echographic multi-spectrometry (REMS) and machine learning (ML) technologies as recently developed tools for the screening and diagnosis of OP.

Medline databases (PubMed) were searched using the following keywords for studies published in 2022: "Gut microbiota AND osteoporosis", "Radio frequency Echographic Multi Spectrometry", "REMS", "Machine Learning AND Osteoporosis".

The relationship between gut microbiota and bone: do we have solid evidence?

The microorganisms that inhabit the human intestine are known as the gut microbiota, and their collective genome is called gut microbiome. The gut microbiota consists of over 10–100 trillion microbes (bacteria, archaea, viruses, fungi, protozoa and eukaryotes; their density and composition vary throughout the intestinal tract, the colon harbouring the most) that encode more than 3.3 million genes (2, 3). *Firmicutes* and *Bacteroides* phyla represent more than 90% of the intestinal microbiota (4). It has been well recognised that this enormous symbiotic population has multiple local effects, such as enhancing the extraction of energy from foods, increasing absorption of nutrients, helping in immune system development, and preventing the colonisation and invasion by pathogens. In addition to these local effects, it has been recently supposed that the gut microbiota may play a role in the systemic regulation of some physiological and pathological processes related to human health. For example, short-chain fatty acids (SCFAs) are metabolites of dietary fibres and are produced by microbiota in the large intestine; SCFAs seem to exert numerous effects such as improving calcium absorption, suppressing appetite, improving glucose tolerance, and affecting pro- and anti-inflammatory properties of immune cells. Microbiota has been reported also to influence the disease activity in some rheumatic conditions (5-7). Recent studies on animal models have reported that the gut microbiota may also contribute to the regulation of bone metabolism, which ignited enthusiasm

about the possibility of preventing bone loss by modulating the gut microbiota and even treating OP by mean of fecal microbiota transplantation (8). It is beyond the scope of this chapter to analyse the multiple mechanisms by which this may happen: the reader can find extensive reviews published in 2022 that examined the suggested connections between the gut microbiota and bone tissue (9-16). Despite hundreds *in vitro* or animal studies illustrating an influence of the gut microbiota/microbioma on the skeleton, there are few studies involving humans. Therefore, the aim of this chapter is to provide a brief report on human studies published in 2022 which investigated the relationship between gut microbiota and bone, and to summarise their results in synthetic conclusion remarks.

Knowledge to date: human studies

The study that first examined the effects on bone of a probiotic was published by Nilsson in 2018 (17). In this proof of concept, double-blind, placebo-controlled study, women (mean age 76 years) with low BMD were randomised to receive orally 10^{10} colony-forming units of *Lactobacillus Reuteri* 6475 daily or placebo for 12 months. Those receiving the probiotic supplement had a reduced loss of volumetric BMD at the tibia (assessed by high-resolution peripheral quantitative computed tomography) compared to those receiving placebo: mean relative change -0.83% (95% CI -1.47% to -0.19%) *vs*. -1.85% (95% CI -2.64% to -1.07%). Furthermore, the same authors in 2022 (18) investigated factors that could influence the response to the probiotic. They selected 20 women with the highest changes (good responders) and 20 with the lowest changes (poor responders) in the tibial BMD from the previous study and characterised the gut microbiome composition and function as well as serum metabolome in both groups. They found that gene richness of the gut microbiota was significantly higher ($p<0.01$) and inflammatory state was significantly improved ($p<0.05$) in the good responders at the end of the 12-months daily supplementation. In particular, the good responder group to *Lactobacillus Reuteri* showed decreased levels of serum inflammation marker ultrasensitive C-reactive protein, which has been reported to be associated to higher BMD (19, 20). Moreover, detrimental changes observed in the gut microbiota of poor responders, including the enrichment of *E.Coli* and its biofilm formation, were lessened in the good responders to *L. Reuteri*; and several SCFAs-producing bacterial species, including *C. Acetobutylicum*, *A. Fermentans*, *A. Muciniphila*, *C. Catus* and *R. Bicirculans* were more abundant in the good responder group than in the poor responder group at 12 months. In summary, *L. Reuteri* supplementation could prevent a deterioration of the gut microbiota and inflammatory status, which may explain the small but significant effect reported on bone density.

SCFAs could influence bone formation. This has been suggested by a study (21) that investigated whether SCFAs are absorbed by osteoblasts and influence early osteoblastic differentiation using a pre-osteoblast murine cell line. In that model, acetate and propionate upregulated the osteoblast differentiation marker alkaline phosphatase; acetate also regulated alkaline phosphatase mRNA expression. If these results were confirmed in human studies, acetate and propionate could be useful for promoting bone formation.

The positive effects of *Lactobacilli* probiotics on bone were confirmed by other randomised, double-blind, placebo-controlled studies in humans (22-24). However, non-significant changes in bone density were observed in another RCT comparing probiotics to placebo (25).

In 2022, Orwoll *et al*. (26) published the results of a study aimed at examining the associations of the fecal microbiome with measures of bone density, microarchitecture, and strength. They obtained stool samples from 831 partecipants in a longitudinal observational large study of community dwelling older men, aged 78 to 98 years; they performed 16S rRNA amplicon sequencing and tested for association between the abundance of microbial genera and bone measures obtained with dual-energy x-ray absorptiometry and high-resolution peripheral quantitative computed tomography (at distal radius, distal and diaphyseal tibia). The abundance of 4 bacterial genera were found to be weakly associated with the bone parameters selected (false discovery rate ≤0.05): *Anaerofilum* (with lower radial and tibial density), *Methanomassiliicoccus* (greater distal cortical porosity), *Ruminocclostridium 9* (with less distal tibial cortical porosity), and *Tyzzerella* (with greater tibial density measures). Also, *Lactobacillus* and *Streptococcus* were both associated with worse bone measures at radial and tibial sites, but only when a less strict criterion (false discovery rate ≤0.1) was adopted. As the Authors themselves recognise, these results must be interpreted with caution: the magnitudes of associations were not large, functional follow-up of the association is lacking, statistical power is low, findings relative to *Lactobacillus* are in contrast with previous findings. We find appropriate the authors' conclusion that "larger cohorts of men and women over wider age ranges and/or more causally incisive methods" are needed. An editorial about the study of Orwoll brilliantly lists the difficulties in conducting down-to-earth studies in this field (27).

Among the many molecules derived from gut microbiota that may influence human health, trimethylamine N-oxide (TMAO) may adversely affect bone health by inducing oxidative stress (28). To test this hypothesis, Elam *et al*. (29) assessed TMAO plasma levels and hip fractures incidence during up to 26 years of follow-up in 5019 adults aged ≥65 years; total hip BMD was assessed by dual-energy x-ray absorptiometry in a subset of patients (n=1400). TMAO was not significantly associated with hip fracture: after controlling for covariates, hazard ratios per TMAO doubling were 1.00 (95% CI 0.92, 1.09) in women and 1.12 (0.95–1.33) in men. TMAO was also not associated with total hip BMD. These results are sharply in contrast with previous small longitudinal, cross-sectional, or retrospective studies (30-32).

The relationship between gut microbiota and bone is far from being clari-

fied. We eagerly await the results of the Prune study (33), a randomised controlled trial that assessed the effects on bone density, bone structure and strength of a 12-month daily prune consumption in postmenopausal women, which has the potential of manipulating the gut microbiota.

Take-home messages

- The gut microbiota may influence the bone (8-16).
- Probiotic supplementation may attenuate bone loss in postmenopausal women, but larger and long-term studies are needed (18-20).
- To date, large studies failed to find a sound relation between gut microbiome or its products and bone density or fractures. Currently there is no scientific basis to include gut microbiota modifications in our treatment strategy of OP (29-32).

Radiofrequency echographic multi-spectrometry (REMS): overcoming the limits of DXA scan

Dual-energy x-ray absorptiometry (DXA) scan at the femur and lumbar spine has traditionally been the gold standard method for the diagnosis of OP (34). Despite being widely available and providing high precision and reproducibility, DXA scan presents several limitations, most importantly the use of x-rays and the overestimation of BMD in patients with spinal osteoarthritis, previous vertebral fractures, or aortic calcifications (35), potentially resulting in misdiagnosis and undertreatment. A recent breakthrough in this regard is represented by REMS, which is a radiation-free tool analysing native ultrasound (US) waves acquired at lumbar vertebrae and/or femur. In contrast with conventional ultrasonography, REMS analyses native unfiltered US waves, thereby providing comprehensive information related to bone quality and quantity and obtaining T- and Z-scores through the comparison with age-, sex-, BMI- and site-matched reference curves created for healthy and pathologic bone tissue. REMS has been validated in mono- and multicentric studies as a tool for the diagnosis of OP (36-38), and it was demonstrated that it had a higher sensitivity than DXA in the detection of female subjects prone to fragility fractures in a follow-up period of 5 years (39). Furthermore, it was demonstrated that T-scores at the lumbar spine obtained through REMS in patients with osteoarthritis (OA) are significantly more reliable for the detection of OP in this category of patients, as compared to DXA scan (40). An Italian study published in 2022 supported these findings by showing that in a cohort of 159 postmenopausal women (66.211.6 years) with previous vertebral fractures or lumbar spine OA, REMS classified as "osteoporotic" a larger percentage of patients with respect to DXA (35.1% *vs*. 9.3%, respectively, in the OA subjects; 58.7% *vs*. 23.3%, respectively, in the subjects with fractures) (41). Therefore, the capability to overcome artifacts such as OA and fractures, along with the absence of radiation and reduced costs, may lead to the use of REMS in general population screenings (37). Indeed, REMS can be employed even in subsets of patients for which DXA is not recommended, *e.g*. pregnant women, breast-feeding women and children (42). For instance, in an exploratory Italian prospective observational study using REMS on 78 pregnant women with uncomplicated pregnancy at 39.1±1.5 weeks have been found to have significantly lower femoral neck BMD values compared to a matched control group of non-pregnant women (0.769±0.094 g/cm^2 *vs*. 0.831±0.101 g/cm^2, p=0.0001), showing that REMS may be used to monitor pregnant women with OP or related risk factors (43).

Recently, REMS has been evaluated on patients with diseases increasing the risk for OP and fragility fractures. A study by Fassio *et al*. showed a promising agreement (Cohen's κ correlation coefficient 0.663, p<0.01) between DXA- and REMS-derived BMD values for the worst site considered and in the consequent fracture risk assessment in a group of 41 patients affected by chronic kidney disease (CKD) receiving peritoneal dialysis (44). A recent study involving 90 female patients with type 2 diabetes mellitus (T2DM) showed that REMS was more reliable than DXA at diagnosing OP in this population (47% *vs*. 28%, respectively). Furthermore, lower values of BMD at the lumbar spine by REMS were significantly associated with a history of major fragility fractures in the T2DM population, whereas BMD by DXA were not (45). Another study found that in a cohort of 50 patients with anorexia nervosa, the subjects with previous vertebral fragility fractures presented lower values of BMD at total hip and lumbar spine by DXA and by REMS with respect to those without history of fractures; however, the difference was significant only for BMD at total hip as measured by REMS (46). However, it should be stated that there have been cases for which REMS was not as accurate as in the previously mentioned studies. For example, Lalli *et al*. assessed the accuracy of REMS in comparison with DXA in disuse-related OP in patients with spinal cord injury and found that REMS overestimated BMD at femoral neck and total hip in this category of patients, possibly due to the atrophy and myosteatosis of the iliopsoas muscle which may influence the ultrasound propagation between the probe and the femoral neck. However, the low number of patients warrant larger studies for confirmation (47).

Considering the high incidence of fragility fractures in the general population aged over 50 and the consequent significant economic burden for the healthcare systems (48), an accurate estimation of fracture risk is crucial and widely used tools in clinical practice for this purpose include FRAX, Garvan, QFracture, Fra-HS and De-FRA (49). REMS has been employed for the development of another tool, called the Fragility Score (FS), which has been shown to have slightly better performance than DXA in discriminating patients with previous fractures (50). Interestingly, a prospective study involving 1989 patients recently demonstrated that FS is more capable of predicting fracture risk in both female and male subjects as compared to BMD T-score values obtained by either DXA or REMS (area under the curve (AUC) for lumbar spine adjusted for age and BMI: FS 0.715 *vs*. REMS BMD T-score

0.636, p=0.02, and *vs*. DXA BMD T-score 0.603, p=0.001; AUC for femur adjusted for age and BMI: FS 0.735 *vs*. REMS BMD T-score 0.568, p=0.05, and *vs*. DXA BMD T-score 0.472, p=0.0003). Furthermore, major osteoporotic fracture risk estimation by FS is provided over a 5-year period, as compared to the longer 10-year timeframe covered by tools such as FRAX (51).

Take-home messages

- REMS is a portable and radiation-free tool which has shown high accuracy in the diagnosis of OP. As opposed to DXA, REMS allows the diagnosis of OP in patients with lumbar spine OA, vertebral fractures, and extensive aortic calcifications (38, 40).
- REMS proved excellent in cohorts of patients with CKD (44), T2DM (45), and anorexia nervosa (46). Further studies are required to assess its accuracy in patients with disuse-related OP related to spinal cord injury (47).
- The REMS-based Fragility Score (FS) is a reliable indicator for the estimation of major osteoporotic fracture risk over a 5-year period (50-51).

Use of machine learning tools for the diagnosis and prevention of osteoporosis

Machine learning (ML) is a scientific discipline that focuses on how computers learn from data and become capable of building statistical models from massive datasets (52). ML technologies have recently demonstrated that they can also play a major role from a clinical perspective (53-55). Such technologies allow the creation of predictive models (*e.g*. logistic regression, artificial neural networks, eXtreme gradient boosting, support vector machine, stacking, random forest) which can be used to predict any range of outputs. Conventionally, ML is classified into supervised learning and unsupervised learning. The former approach is generally used for estimating risk and for creating automated models useful for interpreting instrumental examinations (*e.g*. evaluations of an EKG or a lung computed tomography), whereas the latter is used to search for the presence of specific patterns within a large amount of data (52). The advantages of ML include the ability to analyse different types of data and its potential for time- and cost-effectiveness (56-57). In the medical literature, these tools have been used in various fields, *e.g*. to predict renal flares occurrence after 5 years of remission in patients with lupus nephritis, to describe phenotypes of extra-renal flares (58-59) and radiographic progression in axial spondyloarthropathies (60), and for genomic classification or subtyping in oncology (61).

As previously mentioned, DXA scan is currently the most widely used tool for assessing fracture risk and diagnosing OP, and algorithms such as FRAX or DeFRA efficiently estimate fracture risk (62-65). However, these conventional tools consider a limited number of variables and are used indiscriminately for all patients, often not allowing a weighted risk estimation for different subgroups of diseases (66). Interestingly, ML techniques have been recently investigated for the estimation of fracture risk and for the diagnosis of OP from other imaging tools performed by the patient for other reasons. For example, in the work of Jang *et al*., the deep learning model "OsPor-screen" was trained in a supervised learning manner to recognise OP through retrospective analysis of chest radiographs, yielding promising results. A total of 13,026 chest x-rays and DXAs of individuals between 40 and 90 years of age were analysed, equally divided between "osteoporosis", "osteopenia" and "normal findings". The model was first trained to recognise osteoporosis patients belonging to the "Health Screening and Promotion Centre of Asan Medical Centre" cohort and was subsequently externally validated with the "Asan osteoporosis cohort dataset". The ML model was shown to analyse an x-ray in less than 4 seconds with a sensitivity of 86.2% and specificity of 74.2% in recognising OP in the external validation. This study supports the use of the "OsPor-screen" model as a cost-effective method for opportunistic automated screening of patients with OP in clinical settings and without exposing the patient to additional radiation (67). Similar studies found that ML models were capable of screening for OP based on the analysis of lumbosacral spine and hip radiographs or orthopantomography (68-70). In fact, the presence of OP is often reported by the radiologist as a collateral finding on radiographs, thus being subject to interindividual variability. A similar problem occurs for the estimation of vertebral fractures with Genant semi-quantitative method; in this regard, ML technologies are also useful in the creation of automated screening tools for vertebral fracture assessment and identification of risk factors for refractures (71-72). Indeed, the decision-tree-based model "LightGBM" designed by Microsoft Research Asia to clarify the relevant characteristics that determine refracture after surgically treated fragility fractures has been employed in a study involving 7000 patients, identifying rheumatoid arthritis (RA) and CKD as potential predictors of refracture. In fact, in the study of Shimizu *et al*., the "LightGBM" model was shown to be moderately accurate in predicting refractures, with an AUC of approximately 0.75. Furthermore, when compared to other ML models such as Artificial Neural Networks, "LightGBM" achieved a higher accuracy, suggesting the superiority of decision-tree-based models trained on table data, in this clinical context (72). ML models have also been tested in the context of opportunistic OP screening on images obtained from computed tomography (CT) scans of the chest and abdomen, demonstrating high specificity and sensitivity for OP assessment. Besides, it is also possible to integrate data from CT images with OP risk factors and routine laboratory tests data to build a hierarchical model to identify individuals with OP, as an alternative method to DXA. Liu *et al*. proposed a hierarchical model with three layers: a first layer consisting of only demographic characteristics, a second layer with only clinical data, and a third layer of CT images that partially or completely included the spine. Data of 2210 patients over age 40 were collected ret-

rospectively, then six ML algorithms were used as classifiers to discriminate individuals between osteoporotic and non-osteoporotic; the results showed that the hierarchical model based on logistic regression had better performances, with an area under the receiver operating characteristic curve of 0.818, 0.838 and 0.962 for the three layers, respectively (73-74).

ML technologies can also be employed for the interpretation of complex and multifaceted information, such as that provided by high resolution peripheral quantitative computed tomography (HRpQCT), an imaging technique assessing trabecular and cortical microstructure of bone (75). In the work of Lu *et al*., an automatic high-performance diagnostic algorithm was proposed; it used various inputs (clinical data, bone mineral density measured at the femoral neck, and data from HR-pQCT images of the tibia) to discriminate between patients with or without previous fragility fractures (76). The amount of information provided by HRpQCT, together with the computational capability of ML methods, allowed the identification of bone microarchitecture phenotypes. Whittier *et al*. employed "fuzzy c-means clustering", an unsupervised ML method, which was able to identify three different clusters of bone microarchitecture: low-density, low-volume and healthy bone. Bone phenotypes were identified using cluster analysis and characteristics selected for clustering included height (value used as a surrogate for long bone length) and some HR-pQCT parameters measured at the radius and ulna. According to the authors, the low-density and low-volume phenotypes were those most associated with fragility fractures with a hazard ratio of 2.96 and 2.95, respectively. Therefore, this study suggests the utility of deep learning in assessing fracture risk associated with intrinsic phenotypic characteristics of bone, which DXA is unable to capture (77).

ML techniques are also useful in predicting the fracture risk of certain subpopulations of patients, such as in the case of elderly-onset RA (EORA) or breast cancer. For example, it has been shown that especially the random forest classifier model can accurately predict the occurrence of fractures in patients with RA and OP. In addition, Ji *et al*. constructed three models to predict OP, fragility fractures and survival in breast cancer patients, showing superiority over the FRAX and OSTA (Osteoporosis Self-assessment Tool for Asians) algorithms and thus identifying a new approach for screening this population at risk (78-79).

In conclusion, in the scientific literature, studies related to ML in the context of OP are increasing in number and depth, suggesting a growing interest in this field. The progressive refinement of ML technologies will hopefully allow their use in the daily clinical evaluation of the OP patient.

Take-home messages

- Machine Learning (ML) has emerged as a powerful tool in the clinical setting for creating predictive models that can be used to analyse different types of data. Relevant advantages include time- and cost-effectiveness (53-57).
- ML techniques are being used for the diagnosis and estimation of fracture risk in osteoporosis, and can use information from instrumental examinations already performed, providing a non-invasive method for screening and early detection of the disease (67-70).
- ML models are useful for automated screening tools for vertebral fracture assessment and identification of risk factors for refractures. Decision-tree-based models have been used for the prediction of refractures, as demonstrated by the "LightGBM" model. ML models can also be tested for opportunistic osteoporosis screening on images obtained from CT scans of the chest and abdomen (71-74).

References

1. FIGLIOMENI A, SIGNORINI V, MAZZANTINI M: One year in review 2018: progress in osteoporosis treatment. *Clin Exp Rheumatol* 2018; 36(6): 948-58.
2. QIN J, LI R, RAES J *et al*.: A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; 464(7285): 59-65. https://doi.org/10.1038/nature08821
3. RINNINELLA E, RAOUL P, CINTONI M *et al*.: What is the healthy gut microbiota composition? a changing ecosystem across age, environment, diet, and diseases. *Microorganisms* 2019; 7(1): 14. https://doi.org/10.3390/microorganisms7010014
4. ECKBURG PB, BIK EM, BERNSTEIN CN *et al*.: Diversity of the human intestinal microbial flora. *Science* 2005; 308(5728): 1635-8. https://doi.org/10.1126/science.1110591
5. YASAR BILGE NS, PÉREZ BROCAL V, KASIFOGLU T *et al*.: Intestinal microbiota composition of patients with Behçet's disease: differences between eye, mucocutaneous and vascular involvement. The Rheuma-BIOTA study. *Clin Exp Rheumatol* 2020; 38 (Suppl. 127) (5): S60-68.
6. SELVANDERAN SP, GOLDBLATT F, NGUYEN NQ, COSTELLO SP: Faecal microbiota transplantation for Clostridium difficile infection resulting in a decrease in psoriatic arthritis disease activity. *Clin Exp Rheumatol* 2019; 37(3): 514-15.
7. SCHLESINGER N, BRUNETTI L, ANDROULAKIS IP: Does seasonality of the microbiota contribute to the seasonality of acute gout flare? *Clin Exp Rheumatol* 2022; 40(9): 1793-800. https://doi.org/10.55563/clinexprheumatol/hdtge7
8. ZHANG YW, CAO MM, LI YJ *et al*.: Fecal microbiota transplantation as a promising treatment option for osteoporosis. *J Bone Miner Metab* 2022; 40(6): 874-89. https://doi.org/10.1007/s00774-022-01375-x
9. AWUTI K, WANG X, SHA L, LENG X: Exploring the regulatory mechanism of osteoporosis based on intestinal flora: A review. *Medicine* (Baltimore) 2022; 101(52): e32499. https://doi.org/10.1097/MD.0000000000032499
10. ZEMANOVA N, OMELKA R, MONDOCKOVA V, KOVACOVA V, MARTINIAKOVA M: Roles of gut microbiome in bone homeostasis and its relationship with bone-related diseases. *Biology* (Basel) 2022; 11(10): 1402. https://doi.org/10.3390/biology11101402
11. BHARDWAJ A, SAPRA L, TIWARI A, MISHRA PK, SHARMA S, SRIVASTAVA RK: Osteomicrobiology: the nexus between bone and bugs. *Front Microbiol* 2022; 12: 812466. https://doi.org/10.3389/fmicb.2021.812466
12. CHEN Y, WANG X, ZHANG C, LIU Z, LI C, REN Z: Gut microbiota and bone diseases: a growing partnership. *Front Microbiol* 2022; 13: 877776. https://doi.org/10.3389/fmicb.2022.877776
13. HE Y, CHEN Y: The potential mechanism of the microbiota-gut-bone axis in osteoporosis: a review. *Osteoporos Int* 2022; 33(12): 2495-506. https://doi.org/10.1007/s00198-022-06557-x
14. RASTOGI S, SINGH A: Gut microbiome and human health: Exploring how the probiotic genus *Lactobacillus* modulate immune responses. *Front Pharmacol* 2022; 13: 1042189. https://doi.org/10.3389/fphar.2022.1042189
15. ARMET AM, DEEHAN EC, O'SULLIVAN AF *et al*.: Rethinking healthy eating in light of the gut microbiome. *Cell Host Microbe* 2022; 30(6): 764-85. https://doi.org/10.1016/j.chom.2022.04.016
16. DE SIRE A, DE SIRE R, CURCI C, CASTIGLIONE F, WAHLI W: Role of dietary supplements

and probiotics in modulating microbiota and bone health: the gut-bone axis. *Cells* 2022; 11(4): 743. https://doi.org/10.3390/cells11040743
17. NILSSON AG, SUNDH D, BÄCKHED F, LORENTZON M: Lactobacillus reuteri reduces bone loss in older women with low bone mineral density: a randomized, placebo-controlled, double-blind, clinical trial. *J Intern Med* 2018; 284(3): 307-17. https://doi.org/10.1111/joim.12805
18. LI P, JI B, LUO H, SUNDH D, LORENTZON M, NIELSEN J: One-year supplementation with Lactobacillus reuteri ATCC PTA 6475 counteracts a degradation of gut microbiota in older women with low bone mineral density. *NPJ Biofilms Microbiomes* 2022; 8(1): 84. https://doi.org/10.1038/s41522-022-00348-2
19. DE PABLO P, COOPER MS, BUCKLEY CD: Association between bone mineral density and C-reactive protein in a large population-based sample. *Arthritis Rheum* 2012; 64(8): 2624-31. https://doi.org/10.1002/art.34474
20. KOH JM, KHANG YH, JUNG CH *et al*.: Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. *Osteoporos Int* 2005; 16(10): 1263-71. https://doi.org/10.1007/s00198-005-1840-5
21. KONDO T, CHIBA T, TOUSEN Y: Short-chain fatty acids, acetate and propionate, directly upregulate osteoblastic differentiation. *Int J Food Sci Nutr* 2022; 73(6): 800-8. https://doi.org/10.1080/09637486.2022.2078285
22. JANSONN PA, CURIAC D, LAZOU AHRÉN I *et al*.: Probiotic treatment using a mix of three *Lactobacillus* strains for lumbar spine bone loss in postmenopausal women: a randomized, double-blind, placebo-controlled, multicentre trial. *Lancet Rheumatol* 2019; 1: e154-e162. https://doi.org/10.1016/S2665-9913(19)30068-2
23. TAKIMOTO T, HATANAKA M, HOSHINO T *et al*.: Effect of *Bacillus subtilis* C-3102 on bone mineral density in healthy postmenopausal Japanese women: a randomized, placebo-controlled, double-blind clinical trial. *Biosci Microbiota Food Health* 2018; 37(4): 87-96. https://doi.org/10.12938/bmfh.18-006
24. LAMBERT MNT, THYBO CB, LYKKEBOE S *et al*.: Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a randomized controlled trial. *Am J Clin Nutr* 2017; 106(3): 909-20. https://doi.org/10.3945/ajcn.117.153353
25. JAFARNEJAD S, DJAFARIAN K, FAZELI MR, YEKANINEJAD MS, ROSTAMIAN A, KESHAVARZ SA: Effects of a multispecies probiotic supplement on bone health in osteopenic postmenopausal women: a randomized, double-blind, controlled trial. *J Am Coll Nutr* 2017; 36(7): 497-506. https://doi.org/10.1080/07315724.2017.1318724
26. ORWOLL ES, PARIMI N, WIEDRICK J *et al*.: Analysis of the associations between the human fecal microbiome and bone density, structure, and strength: the Osteoporotic Fractures in Men (MrOS) Cohort. *J Bone Miner Res* 2022; 37(4): 597-607. https://doi.org/10.1002/jbmr.4518
27. LI R, MEDINA-GOMEZ C, RIVADENEIRA F: Down-to-earth studies of the gut microbiome in bone health and disease. *J Bone Miner Res* 2022 Apr;37(4):595-596. https://doi.org/10.1002/jbmr.4547
28. KE Y, LI D, ZHAO M *et al*.: Gut flora-dependent metabolite Trimethylamine-N-oxide accelerates endothelial cell senescence and vascular aging through oxidative stress. *Free Radic Biol Med* 2018; 116: 88-100. https://doi.org/10.1016/j.freeradbiomed.2018.01.007 Erratum in: *Free Radic Biol Med* 2018;129: 608-10.
29. ELAM RE, BŮŽKOVÁ P, BARZILAY JI *et al*.: Trimethylamine N-oxide and hip fracture and bone mineral density in older adults: the cardiovascular health study. *Bone* 2022; 161: 116431. https://doi.org/10.1016/j.bone.2022.116431
30. LIN H, LIU T, LI X, GAO X, WU T, LI P: The role of gut microbiota metabolite trimethylamine N-oxide in functional impairment of bone marrow mesenchymal stem cells in osteoporosis disease. *Ann Transl Med* 2020; 8(16): 1009. https://doi.org/10.21037/atm-20-5307
31. LIU Y, GUO YL, MENG S *et al*.: Gut microbiota-dependent trimethylamine N-oxide are related with hip fracture in postmenopausal women: a matched case-control study. *Aging* (Albany NY). 2020; 12(11): 10633-41. https://doi.org/10.18632/aging.103283
32. ZHOU T, HEIANZA Y, CHEN Y *et al*.: Circulating gut microbiota metabolite trimethylamine N-oxide (TMAO) and changes in bone density in response to weight loss diets: The POUNDS Lost Trial. *Diabetes Care* 2019; 42(8): 1365-71. https://doi.org/10.2337/dc19-0134
33. DE SOUZA MJ, STROCK NCA, ROGERS CJ *et al*.: Rationale and study design of randomized controlled trial of dietary supplementation with prune (dried plums) on bone density, geometry, and estimated bone strength in postmenopausal women: the Prune study. *Contemp Clin Trials Commun* 2022; 28: 100941. https://doi.org/10.1016/j.conctc.2022.100941
34. BAIM S, LESLIE WD: Assessment of fracture risk. *Curr Osteoporos Rep* 2012; 10(1): 28-41. https://doi.org/10.1007/s11914-011-0093-9
35. AMORIM DMR, SAKANE EN, MAEDA SS, LAZARETTI CASTRO M: New technology REMS for bone evaluation compared to DXA in adult women for the osteoporosis diagnosis: a real-life experience. *Arch Osteoporos* 2021; 16(1): 175. https://doi.org/10.1007/s11657-021-00990-x
36. DIEZ-PEREZ A, BRANDI ML, AL-DAGHRI N *et al*.: Radiofrequency echographic multispectrometry for the in-vivo assessment of bone strength: state of the art-outcomes of an expert consensus meeting organized by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). *Aging Clin Exp Res* 2019; 31(10): 1375-89. https://doi.org/10.1007/s40520-019-01294-4
37. CONVERSANO F, FRANCHINI R, GRECO A *et al*.: A novel ultrasound methodology for estimating spine mineral density. *Ultrasound Med Biol* 2015; 41(1): 281-300. https://doi.org/10.1016/j.ultrasmedbio.2014.08.017
38. DI PAOLA M, GATTI D, VIAPIANA O *et al*.: Radiofrequency echographic multispectrometry compared with dual x-ray absorptiometry for osteoporosis diagnosis on lumbar spine and femoral neck. *Osteoporos Int* 2019; 30(2): 391-402. https://doi.org/10.1007/s00198-018-4686-3
39. ADAMI G, ARIOLI G, BIANCHI G *et al*.: Radiofrequency echographic multi spectrometry for the prediction of incident fragility fractures: a 5-year follow-up study. *Bone* 2020; 134: 115297. https://doi.org/10.1016/j.bone.2020.115297
40. CAVALLI L, LOMBARDI FA, PERRONE D, BRANDI ML: SAT0537 The REMS technique is not affected by arthrosis artifact, which can hinder the densitometric recognition of osteoporosis. *Ann Rheum Dis* 2019; 78: 1361.
41. CAFFARELLI C, TOMAI PITINCA MD, AL REFAIE A, DE VITA M, CATAPANO S, GONNELLI S: Could radiofrequency echographic multispectrometry (REMS) overcome the overestimation in BMD by dual-energy x-ray absorptiometry (DXA) at the lumbar spine? *BMC Musculoskelet Disord* 2022; 23(1): 469. https://doi.org/10.1186/s12891-022-05430-6
42. CIARDO D, PISANI P, CONVERSANO F, CASCIARO S: Pulse-echo measurements of bone tissues. techniques and clinical results at the spine and femur. *Adv Exp Med Biol* 2022; 1364: 145-62. https://doi.org/10.1007/978-3-030-91979-5_7
43. DEGENNARO VA, BRANDI ML, CAGNINELLI G *et al*.: First assessment of bone mineral density in healthy pregnant women by means of radiofrequency echographic multi spectrometry (REMS) technology. *Eur J Obstet Gynecol Reprod Biol* 2021; 263: 44-49. https://doi.org/10.1016/j.ejogrb.2021.06.014
44. FASSIO A, ANDREOLA S, GATTI D *et al*.: Radiofrequency echographic multi-spectrometry and DXA for the evaluation of bone mineral density in a peritoneal dialysis setting. *Aging Clin Exp Res* 2023; 35(1): 185-92. https://doi.org/10.1007/s40520-022-02286-7
45. CAFFARELLI C, TOMAI PITINCA MD, AL REFAIE A, CECCARELLI E, GONNELLI S: Ability of radiofrequency echographic multispectrometry to identify osteoporosis status in elderly women with type 2 diabetes. *Aging Clin Exp Res* 2022; 34(1): 121-7. https://doi.org/10.1007/s40520-021-01889-w
46. CAFFARELLI C, AL REFAIE A, DE VITA M *et al*.: Radiofrequency echographic multispectrometry (REMS): an innovative technique for the assessment of bone status in young women with anorexia nervosa. *Eat Weight Disord* 2022; 27(8): 3207-13. https://doi.org/10.1007/s40519-022-01450-2
47. LALLI P, MAUTINO C, BUSSO C *et al*.: Reproducibility and accuracy of the radiofrequency echographic multi-spectrometry for femoral mineral density estimation and discriminative power of the femoral fragility score in patients with primary and disuse-related osteoporosis. *J Clin Med* 2022; 11(13): 3761. https://doi.org/10.3390/jcm11133761
48. STRÖM O, BORGSTRÖM F, KANIS JA *et al*.: Osteoporosis: burden, health care provision and opportunities in the EU: a report pre-

pared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos* 2011; 6: 59-155. https://doi.org/10.1007/s11657-011-0060-
49. ADAMI G, BIFFI A, PORCU G *et al*.: A systematic review on the performance of fracture risk assessment tools: FRAX, DeFRA, FRAHS. *J Endocrinol Invest* 2023 Apr 9. https://doi.org/10.1007/s40618-023-02082-8
50. PISANI P, GRECO A, CONVERSANO F *et al*.: A quantitative ultrasound approach to estimate bone fragility: a first comparison with dual x-ray absorptiometry. *Measurement* 2017; 101: 243-49. https://doi.org/10.1016/j.measurement.2016.07.033
51. PISANI P, CONVERSANO F, MURATORE M *et al*.: Fragility Score: a REMS-based indicator for the prediction of incident fragility fractures at 5 years. *Aging Clin Exp Res* 2023; 35(4): 763-73. https://doi.org/10.1007/s40520-023-02358-2
52. MCMASTER C, BIRD A, LIEW DFL *et al*.: Artificial intelligence and deep learning for rheumatologists. *Arthritis Rheumatol* 2022; 74(12): 1893-905. doi: 10.1002/art.42296
53. KAISSIS GA, MAKOWSKI MR, RÜCKERT D *et al*.: Secure, privacy-preserving and federated machine learning in medical imaging. *Nat Mach Intell* 2020; 2: 305-11.
54. TJOA E, GUAN C: A Survey on Explainable Artificial Intelligence (XAI): Toward Medical XAI. *IEEE Trans Neural Netw Learn Syst* 2021; 32(11): 4793-813. https://doi.org/10.1109/tnnls.2020.3027314
55. GARAFFONI C, ADINOLFI A, BORTOLUZZI A *et al*.: Novel insights into the management of rheumatoid arthritis: one year in review 2022. *Clin Exp Rheumatol* 2022; 40(7): 1247-57. https://doi.org/10.55563/clinexprheumatol/1sjgyr
56. CAREY JJ, CHIH-HSING WU P, BERGIN D: Risk assessment tools for osteoporosis and fractures in 2022. *Best Pract Res Clin Rheumatol* 2022; 36(3): 101775. https://doi.org/10.1016/j.berh.2022.101775
57. LIU L, SI M, MA H *et al*.: A hierarchical opportunistic screening model for osteoporosis using machine learning applied to clinical data and CT images. *BMC Bioinformatics* 2022; 23(1): 63. https://doi.org/10.1186/s12859-022-04596-z
58. CHEN Y, HUANG S, CHEN T *et al*.: Machine learning for prediction and risk stratification of lupus nephritis renal flare. *Am J Nephrol* 2021; 52(2): 152-60.
59. MAFFI M, TANI C, CASCARANO G *et al*.: Which extra-renal flare is "difficult to treat" in systemic lupus erythematosus? A one-year longitudinal study comparing traditional and machine learning approaches. *Rheumatology* (Oxford) 2023 April 24: kead166. https://doi.org/10.1093/rheumatology/kead166
60. GAROFOLI R, RESCHE-RIGON M, ROUX C, VAN DER HEIJDE D, DOUGADOS M, MOLTÓ A: Machine-learning derived algorithms for prediction of radiographic progression in early axial spondyloarthritis. *Clin Exp Rheumatol* 2023; 41(3): 727-34. https://doi.org/10.55563/clinexprheumatol/mm2uzu
61. CHEN D, LIU J, ZANG L *et al*.: Integrated machine learning and bioinformatic analyses constructed a novel stemness-related classifier to predict prognosis and immunotherapy responses for hepatocellular carcinoma patients. *Int J Biol Sci* 2022; 18(1): 360-73. https://doi.org/10.7150/ijbs.66913
62. KANIS JA, HARVEY NC, JOHANSSON H, ODÉN A, LESLIE WD, MCCLOSKEY EV: FRAX Update. *J Clin Densitom* 2017; 20(3): 360-67. https://doi.org/10.1016/j.jocd.2017.06.022
63. ADAMI S, BIANCHI G, BRANDI ML *et al*.: Validation and further development of the WHO 10-year fracture risk assessment tool in Italian postmenopausal women: project rationale and description. *Clin Exp Rheumatol* 2010; 28(4): 561-70.
64. KANIS JA, JOHNELL O, ODEN A, JOHANSSON H, MCCLOSKEY E: FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int* 2008; 19(4): 385-97. https://doi.org/10.1007/s00198-007-0543-5
65. NGUYEN ND, FROST SA, CENTER JR, EISMAN JA, NGUYEN TV: Development of prognostic nomograms for individualizing 5-year and 10-year fracture risks. *Osteoporos Int* 2008; 19(10): 1431-44. https://doi.org/10.1007/s00198-008-0588-0
66. CAREY JJ, CHIH-HSING WU P, BERGIN D: Risk assessment tools for osteoporosis and fractures in 2022. *Best Pract Res Clin Rheumatol* 2022; 36(3): 101775. https://doi.org/10.1016/j.berh.2022.101775
67. JANG M, KIM M, BAE SJ, LEE SH, KOH JM, KIM N: Opportunistic osteoporosis screening using chest radiographs with deep learning: development and external validation with a cohort dataset. *J Bone Miner Res* 2022; 37(2): 369-77. https://doi.org/10.1002/jbmr.4477
68. MAO L, XIA Z, PAN L *et al*.: Deep learning for screening primary osteopenia and osteoporosis using spine radiographs and patient clinical covariates in a Chinese population. Front Endocrinol (Lausanne) 2022; 13: 971877. https://doi.org/10.3389/fendo.2022.971877
69. KONG SH, LEE JW, BAE BU *et al*.: Development of a spine x-ray-based fracture prediction model using a deep learning algorithm. *Endocrinol Metab* (Seoul) 2022; 37(4): 674-83. https://doi.org/10.3803/EnM.2022.1461
70. REN J, FAN H, YANG J, LING H: Detection of trabecular landmarks for osteoporosis prescreening in dental panoramic radiographs. *Annu Int Conf IEEE Eng Med Biol Soc* 2020; 2020: 2194-97. https://doi.org/10.1109/EMBC44109.2020.9175281
71. DONG Q, LUO G, LANE NE *et al*.: Deep learning classification of spinal osteoporotic compression fractures on radiographs using an adaptation of the genant semiquantitative criteria. *Acad Radiol* 2022; 29(12): 1819-32. https://doi.org/10.1016/j.acra.2022.02.020
72. SHIMIZU H, ENDA K, SHIMIZU T *et al*.: Machine learning algorithms: prediction and feature selection for clinical refracture after surgically treated fragility fracture. *J Clin Med* 2022; 11(7): 2021. https://doi.org/10.3390/jcm11072021
73. PICKHARDT PJ, NGUYEN T, PEREZ AA *et al*.: Improved CT-based osteoporosis assessment with a fully automated deep learning tool. *Radiol Artif Intell* 2022; 4(5): e220042. https://doi.org/10.1148/ryai.220042
74. LIU L, SI M, MA H *et al*.: A hierarchical opportunistic screening model for osteoporosis using machine learning applied to clinical data and CT images. *BMC Bioinformatics* 2022; 23(1): 63. https://doi.org/10.1186/s12859-022-04596-z
75. KLOSE-JENSEN R, THERKILDSEN J, BLAVNSFELDT AG *et al*.: Diagnostic accuracy of high-resolution peripheral quantitative computed tomography and x-ray for classifying erosive rheumatoid arthritis. *Rheumatology* (Oxford) 2022; 61(3): 963-73. https://doi.org/10.1093/rheumatology/keab446
76. LU S, FUGGLE NR, WESTBURY LD *et al*.: Machine learning applied to HR-pQCT images improves fracture discrimination provided by DXA and clinical risk factors. *Bone* 2023; 168: 116653. https://doi.org/10.1016/j.bone.2022.116653
77. WHITTIER DE, SAMELSON EJ, HANNAN MT *et al*.: Bone microarchitecture phenotypes identified in older adults are associated with different levels of osteoporotic fracture risk. *J Bone Miner Res* 2022; 37(3): 428-39. https://doi.org/10.1002/jbmr.4494
78. CHEN R, HUANG Q, CHEN L: Development and validation of machine learning models for prediction of fracture risk in patients with elderly-onset rheumatoid arthritis. *Int J Gen Med* 2022; 15: 7817-29. https://doi.org/10.2147/IJGM.S380197
79. JI L, ZHANG W, ZHONG X *et al*.: Osteoporosis, fracture and survival: application of machine learning in breast cancer prediction models. *Front Oncol* 2022; 12: 973307. https://doi.org/10.3389/fonc.2022.973307