Review

The great challenge: bone fragility and environment

M. Mazzantini, G. De Mattia

Rheumatology Unit, Azienda Universitario Ospedaliera di Pisa, Italy.

Maurizio Mazzantini, MD
Giammarco De Mattia, MD

Please address correspondence to:
Maurizio Mazzantini
Reumatologia,
Azienda Universitario
Ospedaliera di Pisa,
via Roma 67,
56126 Pisa, Italy.
E-mail: mmazzant@int.med.unipi.it

Received on March 3, 2024; accepted in revised form on June 7, 2024.

Clin Exp Rheumatol 2024; 42: 1714-1719.

Key words: osteoporosis, fracture, environment, vitamin D, air pollutants

Competing interests: none declared.

ABSTRACT

Osteoporosis is a worldwide common disease characterised by reduced bone mass and increased risk of fractures. Many genetic variants are associated with the disease, but they account for only a small percentage of variance in individual bone mineral density and fragility fracture risk. Only recently have researchers recognised the role of a broad variety of environmental factors in the pathogenesis of osteoporosis, which has led to a further step: how genetic and environmental factors can interact, which is the next frontier in research on bone fragility.

The exposome and the bone

Following the definition given by Wild in 2005 (1), the exposome embraces all life-course environmental exposures (both exogenous and endogenous, including lifestyle factors), from the prenatal period onwards. Unlike the genome, which is highly constant throughout life, the exposome is highly variable and dynamic: food, drugs, gut microbiota (2), oxidative stress, pollutants, climate, ionising radiation, urban environment, physical activity, revenue, and education level (3) are among the numerous factors that contribute to increasing individual phenotypic variability; these factors can affect health and susceptibility to diseases. The interplay between genome and exposome represents an expanding field of research, with the aim of identifying individuals at greatest susceptibility to chronic diseases as well as effective strategies to prevent them. Osteoporosis and fractures are one of the greatest health problems nowadays. There were 568.000 new fragility fractures in Italy in 2019, and this number is expected to increase by 134.000 to 702.000 in 2034 (4); the total direct cost of fractures was €9.45 billion in 2019 (6.0% of healthcare spending), which included a negligible expenditure in pharmacological prevention (€259 million, 2.7% of total expenditure). Given these facts, is of paramount importance to identify the environmental factors that increase fracture risk; slowing down the growth in the number of fractures is one of the greatest challenges of the near future. Fortunately, this challenge is far less daunting now than a few decades ago: research has identified the key role in bone health of environmental factors such as - to cite those more relevant - vitamin D, calcium intake, body weight, body weight changes, physical activity, and pollution. Furthermore, most of these factors are measurable and can serve as markers of risk.

Recognising the vital role of vitamin D in human evolution

In the last two decades, vitamin D has received great attention due to the realising of vitamin D deficiency as a global health issue together with evidence that 1,25-dihydroxyvitamin D3, the hormonally active form of vitamin D, produces a number of skeletal and extra skeletal biological responses. Extra skeletal effects include a role in both innate and adaptative immune system and in muscular function, a possible inhibition of cancer cell progression, favourable effects on the cardiovascular system, and protection against a number of autoimmune disease (5-7). Nowadays vitamin D is very popular as a "bone fixing" agent, but the relationship between vitamin D, calcium homeostasis and bone remodelling turned out to be the last evolutionary function of the vitamin. About 385 million years ago, some species moved from the ocean into land and had to develop a skeleton to support locomotion under gravitational forces (8). In the calcium-rich environment of the sea water (that contains about 400 mg/L of calcium), this transformation would have been

made possible by calcium abundance itself; the calcium-poor conditions on land imposed the requirement of an extremely precise regulation of calcium homeostasis to sustain both calcium-depending muscular and neurologic functions and the bone modelling and remodelling (9). In this process, vitamin D, as well as the parathyroid hormone (PTH), had a vital role. In fact, their primary objective is to maintain serum calcium levels within the normal range, by increasing calcium intestinal absorption, decreasing its renal excretion, and promoting reabsorption of calcium from the bone when necessary. Vitamin D_3 (cholecalciferol), the natural form of vitamin D, is produced in the skin from 7-dehydrocholesterol under proper UV-B irradiation. The skin is the most important source of vitamin D (about 80% of daily requirement) and depends on the intensity of the UV-B irradiation, which in turn is dependent on season and latitude. Vitamin D can also be taken in the diet. However, vitamin D is present in only a few foods (which include fortified dairy products and fish oils), and the amount of vitamin D taken with food does not exceed the 20% of daily requirement (10). The need for an appropriate cutaneous production of vitamin D has been proposed as the explanation of today's North-South gradient in skin colour: when *Homo Sapiens* left equatorial East Africa about 75.000 years ago their skin was dark to protect them from sunburn and cancer caused by the strong equatorial sun. In northern regions, both clothing and northern latitude reduced the amount of vitamin D produced in the skin. Bone disease, reduced potency of the immune system and muscle function impairment may have created an evolutionary pressure, which favoured individuals with a lighter skin (11). This hypothesis, however, in not universally accepted (12). Nowadays, by measuring plasma levels of 25OH-vitamin D, vitamin D deficiency has emerged as a frequent finding in the general population, which certainly contributes to increase bone loss and fracture risk. Assessing individual vitamin D status has become increasingly popular in the recent years, although several guidelines such as those produced by the Italian Osteoporosis Society (13) recommended not performing the 25(OH)D measurement in the general population. Instead, it should be more cost effective to profile the individual risk of hypovitaminosis and then decide to perform the test only in those deemed at risk of osteomalacia; those at risk of hypovitaminosis, such as obese patients, those who do not spend sufficient time outdoors, and institutionalised elderly patients should be supplemented with adequate dose of vitamin D without testing 25OH-vitamin D (13). How to measure reliably the individual risk of vitamin D deficiency? Recently, indirect information regarding vitamin D status in Italian adults has been achieved by means of a 20-item, multiple-choice, validated questionnaire (Evaluation Vitamin D Deficiency Questionnaire, EVIDENCe-Q) exploring the factors affecting the production, intake, absorption, and metabolism of vitamin D (14). In this study the prevalence of severe deficiency (*i.e.* 25OH-vitamin D <10 ng/ml), deficiency (10–20 ng/ml), and insufficiency (20–30 ng/ml) were determined in 22%, 35.3%, and 43.3% of the study population, respectively, confirming the high prevalence of hypovitaminosis in our population; EVIDENCe-Q scores were analysed to 25-OH-D serum levels, showing a statistically significant discriminatory power. Therefore, EVIDENCe-Q can be a screening tool for clinicians in their daily practice to identify subjects at risk of vitamin D deficiency and to avoid inappropriate supplementation and costly blood testing.

Is vitamin D all we need for bone health?

As outlined before, vitamin D was an evolutionary successful hormone that allowed life in a calcium-poor environment. Every day an amount of calcium leaves our body via renal excretion and digestive tract secretion (the so called faecal endogenous calcium), and ideally an equal amount of calcium should be absorbed every day by the small intestine to maintain a neutral balance. Renal handling of glomerular filtrated calcium is tightly regulated by PTH in response to variation of serum calcium; PTH in turn induces activation of vitamin D via 1-α hydroxylation in the proximal renal tubule. The main action of $1,25(OH)_2$-vitamin D is to increase intestinal calcium absorption to restore serum calcium levels (15). This conclusion is based on the evidence that in patients affected with hereditary vitamin D-resistant rickets (HVDRR), which is caused by inactivating mutations in the vitamin D receptor, hypocalcaemia and rickets are reversed when these patients are administered intravenous or high oral calcium (16). More-over, when VDR null mice - an animal model of HVDRR - are fed with a diet high in calcium, rickets and osteomalacia are prevented (17, 18). A second direct action of vitamin D is on osteoblasts. In the situation when dietary calcium intake or the amount of calcium absorbed by the gastrointestinal tract is lower than the amount lost or used for bone remodelling, serum levels of PTH and $1,25(OH)_2D$ increase, which leads to reabsorption of calcium from the bone to maintain normal serum calcium levels. This increased bone resorption during a negative calcium balance is necessary to maintain normocalcaemia, as evidenced by a reduction in serum calcium levels when bone resorption is pharmacologically blocked in the intestinal-specific VDR null mice (19). Vitamin D acts primarily via osteoblast VDR signalling, exerting direct transcriptional control on the expression of RANKL, that binds to its receptor RANK in osteoclast precursors and increases osteoclast formation and action. In vitro co-culture experiments have shown that osteoblast VDR signalling is necessary for $1,25(OH)_2D$-induced osteoclast formation, whereas VDR activity in osteoclasts is not (20, 21). Besides stimulating bone resorption during a negative calcium balance, $1,25(OH)_2D$ also inhibits bone matrix mineralisation (17), by increasing the pyrophosphate levels and osteopontin expression (20), both potent mineralisation inhibitors. Based on this evidence about the role of vitamin D, the conclusion is that to achieving bone health an appropriate dietary intake of calcium is as mandatory as is having normal plasma vitamin D levels. In condition of

low calcium intake, and consequently of low intestinal absorption of calcium, vitamin D will maintain serum levels of calcium in the normal range at the expense of bone. According to the *Rapporto Osmed* (23), in 2022 18.1% of the female population and 5.1% of the male population in Italy were supplemented with vitamin D, 1.5% and 0.2%, respectively, with vitamin D plus calcium, and 0.5% and 0.2%, respectively, with calcium alone. Total expenditure in vitamin D in Italy in 2022 (about €340 million) exceeds the sum of the expenditures for all antifracture therapies together (23). While most patients with fragility fractures goes undiagnosed or unrecognised without receiving a pharmacologic secondary prevention (only about 3% of the Italian female population in 2022 was taking an antifracture therapy), vitamin D is probably largely prescribed to healthy individuals who will never benefit from it in term of fractures (24). Furthermore, available data show that vitamin D is usually prescribed without concomitant calcium supplementation, because of the erroneous belief that vitamin D alone is sufficient to preserve bone mass and to prevent fractures, which is not. In fact, to achieve the beneficial effects on bone of vitamin D, a daily calcium intake of 1-1.2g/day must be guaranteed by either diet or pharmacological supplementation. Moreover, prescribing vitamin D should shift from healthy community dwelling individuals to a more targeted at-risk population (13). More than 30 years ago, the administration of tricalcium phosphate and vitamin D to elderly, institutionalised women caused a reduction of 43% of hip fractures and of 32% of non-vertebral fractures after 18 months of treatment when compared to the effects of double placebo (25). A meta-analysis of randomised controlled trials in postmenopausal women from 50 to 79 years old showed that calcium and vitamin D supplementation significantly reduced the incidence of hip fracture (RR= 0.864; 95% CI: 0.763–0.979) and had an effect on total fracture (RR=0.962; 95% CI: 0.925–1.000; p=0.051) nearly reaching statistical significance (26). Another meta-analysis of randomised controlled trials of vitamin D supplementation alone did not find a reduced risk of any fracture (RR: 1.06; 95% CI, 0.98–1.14) or hip fracture (RR: 1.14; 95% CI, 0.98–1.32). In contrast, a meta-analysis of randomised controlled trials of combined supplementation with vitamin D and calcium found a 6% reduced risk of any fracture (RR: 0.94; 95% CI, 0.89–0.99) and a 16% reduced risk of hip fracture (RR: 0.84; 95% CI, 0.72–0.97) (27). More recently, daily administration of 2000 IU of vitamin D to community dwelling people who were not selected for vitamin D deficiency, low bone mass, or osteoporosis did not reduce fracture incidence in comparison with placebo after a median follow up of 5.3 years (24). This was not unexpected, since 25OH-vitamin D plasma levels were normal (*i.e.* >20 ng/ml) in 84.7% of the samples taken at baseline and only 20% of the participants took calcium supplements. Taken together, these findings strongly suggest that vitamin D exerts beneficial skeletal effects when given with an appropriate calcium intake.

Does body weight have an impact on bone?

Osteocytes are the most numerous cells in bone, accounting for 90%–95% of total bone cells in the adult skeleton (28). They descend from osteoblasts (29) that have been encircled progressively by collagen and calcified matrix. As these cells begin to embed in their *lacunae*, they form dendritic-like processes that create contact and anchoring to similar processes originating from previously embedded osteocytes. When the process of mineralisation is completed, the interconnected cells form what is known as the osteocyte-canalicular network. Osteoblasts and osteoclasts live from days to weeks, but osteocytes can live for years or even decades (29). The known functions of osteocytes are the following: 1. to communicate with osteoclasts and osteoblasts and to link the activities of these two cells; 2. to act as endocrine cells that regulate phosphate reabsorption in the kidney, skeletal muscle function, and insulin secretion in the pancreas; and 3. to regulate bone mechanosensing and mechanotransduction (30-32). Evidence for the mechanosensitive function of osteocytes was revealed when transgenic mice with specific osteocyte ablation failed to respond to unloading-induced bone loss (33). The mechanosensing properties of the osteocyte-canalicular network is the functional basis by which body weight can influence the human skeleton. As a matter of fact, higher body weight is associated with higher bone mineral density (BMD) (34, 35). In a cross-sectional study, by multiple linear regression models adjusted for age, smoking, exercise, alcohol, thiazide use, and oestrogen use, total weight was the most consistent marker of BMD, and it was so more in weight-bearing sites than in the non-weight-bearing sites (34). Moreover, in the Dubbo longitudinal study body mass index was an important predictor of the rate of change at the femoral neck over time in both sexes (36). Higher weight was associated with lower rate of bone loss in the elderly in another study (37). Consequently, actual low weight represents an independent risk factor for osteoporosis and fractures, and it is among the main clinical risk factors used to predict individual fracture risk by means of algorithms such as FRAX. However, the relationship between body weight and bone loss is certainly broader: what are the effects of weight loss on BMD over time? In a prospective cohort study on a large population of women aged 65 and older (38) weight changes were measured over a 5.7 year follow-up, and hip BMD changes were measured over a subsequent 4.4 year follow-up. Weight loss was defined as a decrease of 5% or more from baseline weight, stable weight was defined as less than a 5% change, and weight gain was defined as an increase of 5% or more. The rate of decline in total hip bone density increased from -0.52%/year in women with weight gain to -0.68%/year in women with stable weight to -0.92%/year in women with weight loss (p-value for trend 0.001). Higher rates of hip bone loss were observed in women with weight loss irrespective of body mass index. During a subsequent follow-up of an average 6.6 years after the last assessment of body weight, 6%

of the cohort suffered a first hip fracture. Women with weight loss had 1.8 times the risk (95% CI, 1.43–2.24) of subsequent hip fracture as those with stable or increasing weight. Again, the association between weight loss and increased risk of hip fracture was consistent across categories of body mass index (38). This study and others (39, 40) indicate that weight loss has significant long-term effect on bone density and fractures, not only in elderly women but also in men (41). Consequently, measurement of weight changes, not only of actual weight, should be incorporated into clinical practice to assess fracture risk, and osteoporotic patients should be counselled not to lose weight significantly (*e.g.* more than 5% of their actual weight) unless this is appropriate for other health related issues, which implies nutritional advice and weight monitoring. This point is particularly important since weight regain after weight loss seems not to be associated with lumbar spine or hip bone regain, even when weight loss has been induced by increasing physical activity (40, 42). Exercise could be hypothetically an effective strategy to attenuate bone loss during weight loss. However, randomised controlled studies that tested this hypothesis reported inconsistent findings. A recent meta-analysis (43) included 9 RCTs that compared the changes of BMD in adults with underweight or obesity who underwent a programme of weight reduction through diet alone or in combination with supervised exercise. Diet-induced weight loss plus exercise proved effective in reducing femoral neck bone loss with respect to diet-alone program but failed in attenuating bone loss at the total hip and lumbar spine. While awaiting studies capable to show which types of exercises are more osteogenic, available data suggest that where patient's health requires a body weight reduction this should be achieved by increasing energy consumption instead of by calories restriction. When a significant weight loss is warranted or expected, such as after bariatric surgery, the use of osteoclasts inhibitors such as bisphosphonates could help maintain bone density, but existing data are scarce (44, 45). Preliminary results suggested that even the most potent bisphosphonate, zoledronic acid, was not able to prevent bone loss after bariatric surgery, which testified the potent influence of body weight on the skeleton (45). Conversely, obesity may protect from bone loss in rheumatoid arthritis (46).

Environmental pollution and bone fragility

Exposure to pollutants has long been known to damage several organs and tissues, including the lungs, cardiovascular and central nervous systems. More recently, evidence has emerged of a broader spectrum of damage deriving from pollutants, which includes dysregulation of the immune system (47, 48) and even increased bone loss and fractures. Pollutants may affect the skeleton in different ways. Lead and other heavy metals ingested by diet are concentrated into the bone (49) by binding to hydroxyapatite, where they reside for years, exerting negative effects on bone cells. Air pollutants can absorb and diffuse solar irradiation, thus decreasing ground levels of UVB (50) and affecting cutaneous production of vitamin D. Fine particulate matter (PM) increases the production of RANKL, thus increasing osteoclast formation and activity (51). Exposure to PM of less of 2.5 mm of diameter ($PM_{2.5}$) stimulates alveolar macrophages and bronchial epithelial cells to secrete inflammatory cytokines, such as IL-1β, IL-6, IL-8, and TNF-α (52), which in turn stimulates osteoclast activity. Furthermore, $PM_{2.5}$ induces systemic oxidative damage, which causes impairment of osteoblast formation and survival (53, 54). It is also likely that individuals living in areas of high air pollution spend less time outdoors, which indirectly may cause reduced vitamin D synthesis and reduced physical activity. As a matter of fact, a large epidemiologic study found among white adult males a significant inverse correlation between blood lead level and total hip bone density, after adjustment for age, body mass index, calcium intake, ethanol/tobacco consumption, physical activity, and socioeconomic status; a similar association was found among white females, but the correlation was not significant (55). Comparing by means of ultrastructural microanalysis head femoral biopsies from adult osteoporotic patients who had sustained fragility femoral fractures with those from patients with osteoarthritis or traumatic fractures, a significantly higher prevalence of lead, cadmium and chromium was found in osteoporotic bone than in osteoarthritic or normal bone (56). Lead could affect also the bone of children and adolescents (57). Over the last ten years, extensive data on the negative effect of air pollutants have been collected. In Taiwan, a population-based retrospective cohort study showed an increasing trend in the relationship between air pollutant concentration (CO and NO_2) and the risk of osteoporosis in both men and women. Exposure to the highest level of air pollutants significantly increased by 39% to 89% the risk of osteoporosis with respect to exposure to the lower level, after adjustment for age, sex, insurance fee, urbanisation, and comorbidity (58). A large population-based longitudinal study investigated the association of long-term concentrations of $PM_{2.5}$ and hospital admission for fragility fractures of patients aged 65 years or older living in the Northeast-mid Atlantic US. The risk of bone fracture was greater in areas with higher $PM_{2.5}$ concentrations after controlling for covariates: one interquartile range (4.18 mg/m^3) increase of $PM_{2.5}$ was associated with a 4.1% higher risk of hospital admission for bone fracture (RR: 1.041, 95% CI 1.030–1.051) (59). A large cross sectional Italian study investigated the relationship between long-term exposure to $PM_{2.5}$ and PM_{10} and BMD of women at risk of fracture. After adjustment for age, body mass index, previous fragility fractures, family history of vertebral and hip fractures, menopause, glucocorticoid treatment, comorbidities, and area of residency, women exposed to average levels of PM_{10} above $30mg/m^3$ and those exposed to $PM_{2.5}$ above 25 mg/m^3 had a 15% and 16% higher risk, respectively, of having a T-score of -2.5 or less at any site (60). More recently, a

significant association between osteoporosis risk and air pollutants ($PM_{2.5}$, NO_2 and nitrogen oxide, either singularly or in multiple patterns of combination) has been confirmed in a large study based on data from the UK Biobank (61). This study also assessed whether air pollutants exposure could modify the effects of genetic factors on the risk of osteoporosis and fractures. A genetic risk score (GRS) was created based on a large genome-wide association study of femoral neck bone density: individuals with low GRS exposed to the highest air pollutants had the highest risk of osteoporosis (86.1% greater than that of individuals with low exposure to air pollutants and high GRS) and fracture (44.0% greater than that of individuals with low exposure and high GRS) (61). This is a clear example of how an environmental factor can alter a genetic predisposition to a disease. Since individuals living in areas of high pollution are likely to have increased bone loss and fractures, as either direct or indirect effect of pollutants, this could be considered when profiling the individual risk of fractures and in the decision of pharmacological prevention and treatment of bone loss. The COVID pandemic taught us to wear mask to protect ourselves and the others from infection; indeed, epidemiological data suggest the use of masks outdoor to protect our health every time the concentrations of air pollutants are above the threshold.

References

1. WIND CP: Complementing the genome with an ''Exposome'': the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005;14(8): 1847-50. https://doi.org/10.1158/1055-9965.epi-05-0456
2. MAFFI M, DE MATTIA G, MAZZANTINI M: Osteoporosis and gut microbiota, radiofrequency echografic multispectometry and machine learning: one year in review 2023. *Clin Exp Rheumatol* 2023; 41(7): 1377-83. https://doi.org/10.55563/clinexprheumatol/ma4l1t
3. BALAJ M, HERSON CA, ARONSSON A *et al*.: Effects of education on adult mortality: a global systematic review and meta-analysis. *Lancet Public Health* 2024; 23: S2468-2667. https://doi.org/10.1016/s2468-2667(23)00306-7
4. WILLERS C, NORTON N, HARVEY NC *et al*.: Osteoporosis in Europe: a compendium of country-specific reports. *Arch Osteoporos* 2022;17(1): 23. https://doi.org/10.1007/s11657-021-00969-8
5. HOLICK MF: Vitamin D deficiency. *N Engl J Med* 2007; 357: 266-81. https://doi.org/10.1056/nejmra070553
6. BELLAN M, ANDREOLI L, NERVIANI A *et al*.: Is cholecalciferol a potential disease-modifying anti-rheumatic drug for the management of rheumatoid arthritis? *Clin Exp Rheumatol* 2020; 38: 343-49. https://doi.org/ 10.55563/clinexprheumatol/tdf172
7. CECCHETTI S, TATAR Z, GALAN P *et al*.: Prevalence of vitamin D deficiency in rheumatoid arthritis and association with disease activity and cardiovascular risk factors: data from the COMEDRA study. *Clin Exp Rheumatol* 2016; 34: 984-90.
8. BOUILLON R, SUDA T: Vitamin D: Calcium and bone homeostasis during evolution. *Bonekey Rep* 2014; 3: 480. https://doi.org/10.1038/bonekey.2013.214
9. CARLBERG C: Vitamin D in the context of evolution. *Nutrients* 2022; 14: 3018. https://doi.org/10.3390/nu14153018
10. NUTI R, GENNARI L, CAVATI L *et al*.: Dietary vitamin D intake in Italian subjects: validation of a frequency food questionnaire (FFQ). *Nutrients* 2023; 15: 2969. https://doi.org/10.3390/nu15132969
11. JABLONSKI NG, CHAPLIN G: The evolution of human skin coloration. *J Hum Evol* 2000; 39(1): 57-106. https://doi.org/10.1006/jhev.2000.0403
12. HANEL A, CARLBERG C: Skin colour and vitamin D: an update. *Exp Dermatol* 2020; 29: 864-75. https://doi.org/10.1111/exd.14142
13. BERTOLDO F, CIANFEROTTI L, DI MONACO M *et al*.: Definition, assessment, and management of vitamin D inadequacy: suggestions, recommendations, and warnings from the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS). *Nutrients* 2022; 14: 4148. https:// doi.org/10.3390/nu14194148
14. DE GIUSEPPE R, TOMASINELLI CE, CENA H *et al*.: Development of a short questionnaire for the screening for vitamin D deficiency in Italian adults: the EVIDENCe-Q project. *Nutrients* 2022; 14: 1772. https://doi.org/10.3390/nu14091772
15. CHRISTAKOS S, DHAWAN P, VERSTUYF A, LERLINDEN L, CARMELIET G: Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* 2016; 96: 365-408. https://doi.org/10.1152/physrev.00014.2015
16. HOCHBERG Z, TIOSANO D, EVEN L: Calcium therapy for calcitriol-resistant rickets. *J Pediatr* 1992; 121: 803-8. https://doi.org/10.1016/s0022-3476(05)81919-5
17. AMLING M, PRIEMEL M, HOLZMANN T *et al*.: Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. *Endocrinology* 1999; 140: 4982-87. https://doi.org/10.1210/endo.140.11.7110
18. MASUYAMA R, NAKAYA Y, KATSUMATA S *et al*.: Dietary calcium and phosphorus ratio regulates bone mineralization and turnover in vitamin D receptor knockout mice by affecting intestinal calcium and phosphorus absorption. *J Bone Miner Res* 2003: 18: 1217-26. https://doi.org/10.1359/jbmr.2003.18.7.1217
19. LIEBEN L, MASUYAMA R, TORREKENS S *et al*.: Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. *J Clin Invest* 2012; 122: 1803-15. https://doi.org/10.1172/jci45890
20. TAKEDA S, YOSHIZAWA T, NAGAI Y *et al*.: Stimulation of osteoclast formation by 1,25-dihydroxyvitamin D requires its binding to vitamin D receptor (VDR) in osteoblastic cells: studies using VDR knockout mice. *Endocrinology* 1999; 140: 1005-8. https://doi.org/10.1210/endo.140.2.6673
21. VERLINDEN L, JANSSENS I, DOMS S, VAN HEVEL J, CARMELIET G, VERSTUYF A: VDR expression in osteoclast precursors is not critical in bone homeostasis. *J Steroid Biochem Mol Biol* 2019; 195: 105478. https://doi.org/10.1016/j.jsbmb.2019.105478
22. SHEN Q, CHRISTAKOS S: The vitamin D receptor, Runx2, and the Notch Signaling Pathway cooperate in the transcriptional regulation of osteopontin. *J Biol Chem* 2005; 49: 40589. https://doi.org/10.1074/jbc.m504166200
23. RAPPORTO OSMED 2022, 2023 https://www.aifa.gov.it/uso-dei-farmaci-in-italia
24. LEBOFF MS, CHOU SH, RATLIFF KA *et al*.: Supplemental vitamin D and incident fractures in midlife and older adults. *N Engl J Med* 2022; 387: 299-309. https://doi.org/10.1056/nejmoa2202106
25. CHAPUY MC, ARLOT ME, DUBOEUF F *et al*.: Vitamin D_3 and calcium to prevent hip fractures in elderly women. *N Engl J Med* 1992; 327: 1637-42. https://doi.org/10.1056/nejm199212033272305
26. LIU C, KUANG X, LI K, GUO X, DENG Q, LI D: Effects of combined calcium and vitamin D supplementation on osteoporosis in postmenopausal women: a systematic review and meta-analysis of randomized controlled trials. *Food Funct* 2020: 11: 10817-27. https://doi.org/10.1039/d0fo00787k
27. YAO P, BENNET D, MAFHAM M *et al*.: Vitamin D and calcium for the prevention of fracture. A systematic review and meta-analysis. *JAMA Network Open* 2019; 2(12): e1917789. https://doi:10.1001/jamanetworkopen.2019.17789
28. BONEWALD LF: The amazing osteocyte. *J Bone Min Res* 2011; 26: 229-38. https://doi.org/10.1002/jbmr.320
29. MANOLAGAS SC: Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev* 2000; 21:115-37. https://doi.org/10.1210/edrv.21.2.0395
30. UDA Y, AZA E, SUN N, SHI C, PAJEVIC PD: Osteocyte mechanobiology. *Curr Osteoporos Rep* 2017; 15: 318-25. https://doi.org/10.1007/s11914-017-0373-0
31. QIN L, LUI W, CAO H, XIAO G: Molecular mechanosensors in osteocytes. *Bone Res* 2020; 8: 23. https://doi.org/10.1038/s41413-020-0099-y
32. ROBLING AG, BONEWALD LF: The osteocytes: new insights. *Ann Rev Physiol* 2020; 82: 485-506. https://doi.org/10.1146/annurev-

physiol-021119-034332
33. TATSUMI S, ISHII K, AMIZUKA N *et al*.: Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab* 2007; 5: 464-75. https://doi.org/10.1016/j.cmet.2007.05.001
34. EDELSTEIN SL, BARRETT-CONNOR E: Relation between body size and bone mineral density in elderly men and women. *Am J Epidemiol* 1993; 138: 160-69. https://doi.org/10.1093/oxfordjournals.aje.a116842
35. FELSON DT, ZHANG Y, HANNAN MT, ANDERSSON JJ: Effects of weight and body mass index on bone mineral density in men and women: the Framingham Study. *J Bone Miner Res* 1993; 8: 567-73. https://doi.org/10.1002/jbmr.5650080507
36. JONES G, NGUYEN TV, SAMBROOK PN, KELLY PJ, EISMAN JA: Progressive femoral neck bone loss in the elderly: longitudinal findings from the Dubbo Osteoporosis Epidemiology Study. *BMJ* 1994; 309: 691-95. https://doi.org/10.1002/jbmr.5650080507
37. ENSRUD KE, PALERMO L, BLACK DM *et al*.: Hip and calcaneous bone loss increase with advancing age: longitudinal results from the Study of Osteoporotic Fracture. *J Bone Miner Res* 1995; 10: 1778-87. https://doi.org/10.1002/jbmr.5650101122
38. ENSRUD KE, EWING SK, STONE KL *et al*.: Intentional and unintentional weight loss increase bone loss and hip fracture risk in older women. *J Am Geriatr Soc* 2003; 51: 1740-47. https://doi.org/10.1046/j.1532-5415.2003.51558.x
39. JOHNSON KC, BRAY GA, CHESKIN LJ *et al*.: The effect of intentional weight loss on fracture risk in persons with diabetes: results from the Look AHEAD Randomized Clinical Trial. *J Bone Miner Res* 2017; 32: 2278-87. https://doi:10.1002/jbmr.3214
40. KAMMIRE DE, WALKUP MP, AMBROSIUS WT *et al*.: Effect of weight change following intentional weight loss on bone health in older adults with obesity. *Obesity* 2019; 27: 1839-45. https://doi.org/10.1002/oby.22604
41. ENSRUD KE, HARRISON SL, CAULEY JA *et al*.: Impact of competing risk of mortality on association of weight loss with risk of central body fractures in older men: a prospective cohort study. *J Bone Miner Res* 2017; 32: 624-32. https://doi.org/10.1002/jbmr.3020
42. VILLALON, KL, GOZANSKY WS, VAN PELT RE *et al*.: A losing battle: weight regain does not restore weight loss-induced bone loss in postmenopausal women. *Obesity* 2011; 19: 2345-50. https://doi.org/10.1038/oby.2011.263
43. MESINOVIC J, JANSONS P, ZENGIN A *et al*.: Exercise attenuates bone mineral density loss during diet-induced weight loss in adults with overweight and obesity: a systematic review and meta-analysis. *J Sport Health Sci* 2021; 10: 550-59. https://doi.org/10.1016/j.jshs.2021.05.001
44. FLORES LE, BEAVERS KM, BEAVERS DP *et al*.: Risedronate use may blunt appendicular lean mass loss secondary to sleeve gastrectomy: results from a pilot randomized controlled trial. *CSM Rapid Commun* 2023; 6: 18-25. https://doi.org/10.1002/rco2.72
45. LIU Y, COTÉ MM, CHENEY MC *et al*.: Zoledronic acid for the prevention of bone loss in patients receiving bariatric surgery. *Bone Rep* 2021; 14: 100760. https://doi.org/10.1016/j.bonr.2021.100760
46. LEE SY, JUNG KH, PARK SG, KWON SR, PARK W, LIM MJ: Obesity potentially protects against systemic bone loss in patients with rheumatoid arthritis treated with tumour necrosis factor inhibitors. *Clin Exp Rheumatol* 2021; 39: 125-31. https://doi.org/10.55563/clinexprheumatol/j6zhfm
47. ADAMI G, PONTALTI M, CATTANI G *et al*.: Association between long-term exposure to air pollution and immune-mediated diseases: a population-based cohort study. *RMD Open* 2022; 8: e0022055. https://doi.org/10.1136/rmdopen-2021-002055
48. ZEFT AS, PRAHALAD S, SCHNEIDER R *et al*.: Systemic onset juvenile idiopathic arthritis and exposure to fine particulate air pollution. *Clin Exp Rheumatol* 2016; 34: 946-52.
49. WEDEEN RP: Removing lead from bone: clinical implications of bone lead stores. *Neurotoxicology* 1992; 13: 843-52.
50. BARNARD WF, SAXENA VK, WENNY BN, DELUISI JJ: Daily surface UV exposure and its relationship to surface pollutant measurements. *J Air Waste Manag Assoc* 2003; 53: 237-45. https://doi.org/10.1080/10473289.2003.10466134
51. SAHA H, MUKHERJEE B, BINDHANI B, RAY MR: Changes in RANKL and osteoprotegerin expression after chronic exposure to indoor air pollution as a result of cooking with biomass fuel. *J Appl Toxicol* 2016; 36: 969-76. https://doi.org/10.1002/jat.3275
52. HAN B, XU J, ZHANG Y *et al*.: Associations of exposure to fine particulate matter mass and constituents with systemic inflammation: a cross-sectional study of urban older adults in China. *Environ Sci Technol* 2022; 56: 7244-55. https://doi.org/10.1021/acs.est.1c04488
53. MØLLER P, LOFT S: Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environ Health Perspect* 2010; 118: 1126-36. https://doi.org/10.1289/ehp.0901725
54. TIAN Y, HU Y, HOU X, TIAN F: Impact and mechanism of $PM_{2.5}$ on bone. *Rev Environ Health* 2023. https://doi.org/10.1515/reveh-2023-0024
55. CAMPBELL JR, AUINGER P: The association between blood lead levels and osteoporosis among adults. Results from the third National Health and Nutrition Examination Survey (NHANES III). *Environ Health Perspect* 2006;1 15: 1018-22. https://doi.org/10.1289/ehp.9716
56. SCIMECA M, FEOLA M, ROMANO L *et al*.: Heavy metals accumulation affects bone microarchitecture in osteoporotic patients. *Environ Toxicol* 2017; 32: 1333-42. https://doi.org/10.1002/tox.22327
57. CUI A, XIAO P, HU B *et al*.: Blood lead level is negatively associated with bone mineral density in U.S. children and adolescents aged 9-19 years. *Front Endocrinol* 2022: 13: 928752. https://doi.org/10.3389/fendo.2022.928752
58. CHANG KH, CHANG MY, MUO CH *et al*.: Exposure to air pollution increases the risk of osteoporosis. A nationwide longitudinal study. *Medicine* 2015; 94: e733. https://doi.org/10.1097/md.0000000000000733
59. PRADA D, ZHANG J, COLICINO E *et al*.: Association of air particulate pollution with bone loss over time and bone fracture risk: analysis of data from two independent studies. *Lancet Planet Health* 2017; 1: e337-347. https://doi.org/10.1016/s2542-5196(17)30136-5
60. ADAMI G, CATTANI G, ROSSINI M *et al*.: Association between exposure to fine particulate matter and osteoporosis: a population-based cohort study. *Osteoporos Int* 2022; 33: 169-76. https://doi.org/10.1007/s00198-021-06060-9
61. YU XH, CAO HW, BO L, LEI SF, DENG FY: Air pollution, genetic factors and the risk of osteoporosis: a prospective study in the UK biobank. *Front Public Health* 2023; 11: 1119774. https://doi.org/10.3389/fpubh.2023.1119774