The effect of calcium and vitamin D supplementation on osteoporotic rabbit bones studied by vibrational spectroscopy

Athina Lani · Nikolaos Kourkoumelis ·
Gerasimos Balioukas · Margaret Tzaphlidou

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Abstract Fourier transform infrared spectroscopy is utilized to examine the effects of increased calcium, vitamin D, and combined calcium-vitamin D supplementation on osteoporotic rabbit bones with induced inflammation. The study includes different bone sites (femur, tibia, humerus, vertebral rib) in an effort to explore possible differences among the sites. We evaluate the following parameters: mineral-to-matrix ratio, carbonate content, and non-apatitic species (labile acid phosphate and labile carbonate) contribution to bone mineral. Results show that a relatively high dose of calcium or calcium with vitamin D supplementation increases the bone mineralization index significantly. On the other hand, vitamin D alone is not as effective in promoting mineralization even with high intake. Mature B-type apatite was detected for the group with calcium supplementation similar to that of aged bone. High vitamin D intake led to increased labile species concentration revealing bone formation. This is directly associated with the suppression of pro-inflammatory cytokines linked to induced inflammation. The latter is known to adversely alter bone metabolism, contributing to the aetiopathogenesis of osteoporosis. Thus, a high intake of vitamin D under inflammation-induced osteoporosis does not promote mineralization but suppresses bone resorption and restores metabolic balance.

Keywords FTIR spectroscopy · Calcium · Vitamin D · Induced inflammation · Osteoporosis · Bone composition

1 Introduction

Nutritional deficiencies together with genetic abnormalities and hormonal disorders can severely affect normal bone mineralization. Under these conditions, bone mass and strength are reduced while the overall bone quality deteriorates. Skeleton growth requires large amounts of calcium (and phosphorus), which are supplied to the organism by diet. Three calcium-regulating hormones, namely parathyroid hormone (PTH), calcitriol (1,25-dihydroxy-vitamin D, 1,25(OH)₂D) and calcitonin, are of critical importance affecting calcium and

A. Lani · N. Kourkoumelis (✉) · G. Balioukas · M. Tzaphlidou
Department of Medical Physics, Medical School, University of Ioannina, Ioannina 45110, Greece
e-mail: nkourkou@uoii.gr

phosphorus resources available for the formation of healthy bones. PTH regulates calcium levels and triggers bone formation and resorption; calcitriol is the key circulating vitamin D metabolite and is derived from vitamin D [1] by enzymes in the liver and the kidney and stimulates intestines to absorb calcium and phosphorus; finally, calcitonin inhibits bone breakdown by inactivating osteoclasts, protects against excessive calcium concentration in the blood, and plays a major role in maintaining calcium in bone. Vitamin D insufficiency reduces the absorption of calcium, causing rickets in children and osteomalacia in adults, leading to fractures and bone deformities. The pathogenesis of osteoporosis stems mainly from calcium deficiency and secondary hyperparathyroidism. Inadequate amounts of vitamin D and calcium result in unbalanced bone resorption due to decreased serum levels of calcium and increased levels of PTH.

Calcium is the most abundant mineral in the human body. It is a necessary nutrient and its utter importance is emphasized during all ages either for acquiring optimal bone mass or for preventing mass loss in the elderly [2]. The correlation between calcium intake and the risk of osteoporosis has been studied extensively in the literature but results are inconclusive because some observational and meta-analysis studies confirm a relationship between high calcium intake and the prevention of bone loss and fractures [3–6], while other studies report insignificant or even adverse effects as a result of calcium supplementation [7, 8]. Moreover, Lee et al. suggested that bone mineral acquisition stops as soon as calcium supplementation is withdrawn [9, 10].

The current consensus, however, is that low fractional absorption of calcium is related to increased risk for osteoporotic fractures. Dietary calcium intake and osteoporosis follow a non-linear relationship where osteoporotic findings are associated with very low dairy calcium intake [6, 11]. This is manifested as a threshold level for calcium content below which negative metabolic effects, leading to significant loss of bone mass, are evident.

Hypovitaminosis D is caused by a lack of exposure to UVB rays (sunlight irradiation of skin) and/or by inadequate dietary intake and is directly related to PTH suppression and abnormal bone metabolism. Vitamin D deficiency causes secondary hyperparathyroidism and leads to bone characteristics similar to those of aged, over-mineralized, bone. Upon the surface of heavily mineralized tissue a thick layer of unmineralized osteoid coating is formed [12]. Similarly to calcium intake, the optimal amount of vitamin D intake for musculoskeletal growth remains controversial [13–16], mostly because vitamin D insufficiency cannot be explicitly defined [17]. Large-scale epidemiological studies like the National Health and Nutrition Examination Survey III (NHANES III) concluded that high concentrations of vitamin D do act against fracture risk [18] while others, which have also taken into account an increased number of patients, like the OFELY (Os des Femmes de Lyon) study, failed to prove a significant effect of vitamin D supplements on bone mineral density (BMD) in the long term [19]. The effect of vitamin D action on skeleton condition has recently been re-evaluated exhibiting the broader biological role of vitamin D but the typical one of calcium metabolism and bone homeostasis [20]. In addition, lower levels of vitamin D have been linked to familial longevity [21]. It is not clear, however, if vitamin D supplementation is directly related to the treatment of osteoporosis largely because vitamin D is usually accompanied by pharmacologic treatment (steroids, bisphosphonates, etc.), which can obscure any possible net effect [22, 23]. Nevertheless, animal studies have shown that bisphosphonates' efficiency was reduced under a vitamin D-deprived diet [24].

Treatment with calcium and vitamin D is the cornerstone for the prevention of osteoporosis [25]. The Institute of Medicine has recently reviewed the current knowledge on this subject [26, 27] while the recent analysis of the Fourth and Fifth Korea National Health and Nutrition Examination Survey (KNHANES IV-3, 2009 and KNHANES V-1, 2010) showed that both

calcium intake and lower 1,25(OH)D concentrations were correlated with serum PTH and BMD levels in a statistical sample of 4,662 people with a high prevalence of low calcium intake [28].

The requirements and effects under osteoporotic conditions have not yet been identified unambiguously [29]. The National Osteoporosis Risk Assessment (NORA) study [30] concluded that higher calcium and vitamin D intakes can significantly act against osteoporosis, as defined by BMD, without however reducing the odds of a possible fracture. However, stopping the combined calcium vitamin D supplementation resulted in going back to the pretreatment levels of bone turnover [31].

In this work, we examine the effects of a relatively high-dose supplementation of calcium, vitamin D, and combined calcium-vitamin D, on osteoporotic rabbit bones utilizing Fourier transform infrared (FTIR) spectroscopy. FTIR spectroscopy is able to explore biochemical markers of bone metabolism via the vibrational characteristics of molecular groups belonging both to the organic and the inorganic phase of bone.

2 Materials and methods

2.1 Bone samples

Twenty female New Zealand white rabbits, 8 months of age, were randomly housed into four equal groups. Osteoporosis due to inflammation (IMO) was induced in all animals by injecting magnesium silicate (talcum) subcutaneously according to [32, 33]. The mineralization status of the IMO rabbits was then confirmed through energy-dispersive X-ray spectroscopy measurements; significantly lower Ca/P ratio values indicating induced osteoporosis were detected similarly to previous results [32]. It is known that there is no ideal animal model that can effectively replicate the symptoms of spontaneous human osteoporosis. However, the importance of preclinical studies for preventive or curative treatment for osteoporosis symptoms is also well documented [34–36]. The current gold standard for postmenopausal osteoporosis studies is the ovariectomized rat. Still, this model suffers from severe disadvantages: the bones in rat skeleton exhibit lifelong growth and epiphyseal plate fusion does not occur during sexual maturation; thus, true skeletal maturity is hardly achieved in rodents [37]. Additional drawbacks are the lack of a Haversian system and little to no intracortical remodeling [38, 39]. Skeletally mature, New Zealand white rabbits were selected for this study due to their fast bone turnover and the presence of a Haversian remodeling system. Regular bone formation and resorption is established at 32–36 weeks and the remodeling period lasts approximately 70 days [40]. Progressive negative endosteal balance and positive periosteal accrual are also evident for the long bones of aging rabbits similar to postmenopausal women [35]. In addition, similarly to humans, suboptimal Ca intake by rabbits affects bone mass [35].

The IMO protocol has a considerable effect on the bone mineral phase and it is independent of parathormone (PTH) and vitamin D metabolites. Our experimental procedure consisted of four osteoporotic groups: one group (the control group) was fed a normal diet containing Ca 1.2%–1,000 IU/kg diet, vitamin D3. The other three groups were supplied for 60 days ad libitum access to (i) Ca 1.2%–2,000 IU/kg, vitamin D3, (ii) Ca 2.4%–1,000 IU/kg, vitamin D3 and (iii) Ca 2.4%–2,000 IU/kg, vitamin D3.

The calcium content for group (i) was based on the normal commercial rabbit diet, although it is approximately twice of that recommended by the U.S. National Research Council [41] and approximately eightfold greater than the one recommended for patients with osteoporosis [27]. A recent meta-analysis showed that calcium supplements without vitamin D can increase the

risk of myocardial infarction [42]. Results are contradictory regarding this issue especially when calcium supplements are combined with vitamin D [43–45] while outcomes suggesting that vitamin D can counteract the possible harmful vascular effects of calcium supplements are also inconclusive [46]. In our case, we noticed no signs of cardiovascular events for the treated rabbits. Due to the long half-life of vitamin D accumulation in the tissues, an extreme dose of vitamin D can cause chronic toxic effects [47]. Levels of 2,300–3,000 IU/kg are toxic to rabbits and therefore the vitamin D content for the groups (i) and (iii) was the upper plateau.

Multiple bone sites were selected for this study: cortical sections from the right midshaft of femur, tibia, and humerus and trabecular sections of vertebral rib. Throughout the experiments it was important to ensure minimal pain or discomfort for the animals. Thus, the study was conducted under the approval of the Ioannina University Institutional Animal Care and Use Committee.

2.2 Spectral data collection

FTIR spectra were recorded with a Perkin–Elmer Spectrum GX FTIR system in the 3,200–400 cm^{-1} range with 4 cm^{-1} resolution. For each sample, 12-mm KBr pellets consisting of 10% by weight bone powder were prepared. Baseline correction and normalization were performed prior to data analysis. Savitsky–Golay first and second derivatives marked the precise peak locations. Curve fitting and area integrations were performed with a non-linear least-squares algorithm (Levenberg–Marquardt) with OriginPro (OriginLab Corporation, Northampton, MA). Convergence was accomplished in all cases ($R^2=0.99$). Sub-bands are reported as the percentage of the integrated area of the whole IR absorption band.

2.3 Statistical analysis

Statistical significant differences were analyzed with PRISM software (GRAPHPAD software, San Diego, CA, USA) using ANOVA tests with multiple planned comparisons and post hoc Holm–Šidák tests. Brown–Forsythe tests (analyses of variance on deviations from group medians) were utilized to compare data variance among groups. Data variance is reported as mean and standard deviation (SD). Statistical significance was determined at $p \leq 0.05$.

3 Results and discussion

3.1 FTIR spectra. Regions of interest

Typical vibrational bands of a humerus bone sample from groups (i)–(iii) are depicted in Fig. 1.

The fingerprint region represents bone mineral. The very strong absorption band at 1,200–900 cm^{-1} is assigned to the ν_1 (A_1) (symmetric stretching) and ν_3 (T_2) (antisymmetric stretching) normal modes of the apatitic phosphate ion (PO_4^{3-}) [48]. The appearance of the latent ν_1 dipole results in the removal of the degeneracy of the ν_3 mode, showing that the phosphate ion in the biological apatite adopts lower point group symmetry (C_{3v}) than the ideal T_d [48]. This is further confirmed by the weak bands at 957 cm^{-1} (weak shoulder) and 473 cm^{-1} for ν_1 , ν_2 dipoles, respectively. The ν_4 (T_2) (antisymmetric bending) mode appears as a doublet at 560 and 603 cm^{-1} . Carbonate content is manifested as a weak but well-defined absorption band at 871 cm^{-1} attributed to the ν_2 (A_2'') (out of plane bending) vibration. On the other hand, the strong absorption band at 1,664 cm^{-1} represents the amide I functional group

Fig. 1 Typical infrared spectra of bone powder. Absorption bands of interest are indicated. Small differences among groups were evaluated by peak deconvolution. The deconvolution of the $\text{CO}_3^{2-} \nu_2$ contour is shown in the inset

(peptide bond C = O stretching vibration plus C-N stretching vibration of collagen) and it is an appropriate indicator of type I collagen, non-collagenous proteins and water present in bone. The band appearing at $1,549 \text{ cm}^{-1}$ is attributed to the amide II N-H bending and C-N stretching [49].

3.2 Apatitic content

An indirect measurement of BMD can be approximated by the mineral-to-matrix ratio, which is the ratio of the integrated area of the ν_1, ν_3 phosphate absorbance band to the integrated area of the protein amide I absorbance band [50]. This parameter is able to reveal bone hyper- or hypo-mineralization [51] and is found to decrease in osteoporosis [52, 53]. In Fig. 2, we notice a significant increase in the overall bone mineral content ($0.01 \leq p < 0.05$) for both groups (ii) and (iii) for all bone sites compared with the control group. Similarly, there is an increasing trend, albeit not significant ($p=0.11$) regarding the overall variance for group (i). A direct comparison of similar bone sites between the control and the other groups also reveals significant differences for groups (ii) and (iii) while for group (i) only between the tibia and rib sections.

In a recent systematic review and meta-analysis on the effects of low-dose vitamin D supplements on bone mineral density, no evidence was found to support the hypothesis that vitamin D promotes mineralization [54]. Moreover, it has been suggested that vitamin D

Fig. 2 Parameters studied by FTIR spectroscopy for different bone sites and among osteoporotic and treated samples (control, D3: group (i), Ca: group (ii), Ca + D3: group (iii)). SDs plotted as *error bars*. Asterisks denote statistical differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) between the control and the other groups. However, the *dotted columns* (in the “labile acid phosphate” graph) denote that the statistical comparison was done towards this group (Ca) and not towards the control (*faint columns*)

actually inhibits mineralization by increasing local pyrophosphate concentrations [55]. Likewise, severely vitamin D-deficient rats fed with a high-calcium diet had no evidence of rickets (or other pathology) [56] and patients with vitamin D-dependent rickets type 2 were cured by long-term calcium infusion [57]. Our results suggest that high-dose supplementation, specifically when calcium is involved, significantly reduces the osteoporotic effects leading to faster mineral accumulation. In all cases (control samples included), trabecular rib sections feature a significant ($0.01 \leq p < 0.04$) lower mineral-to-matrix ratio in agreement with the slower turnover rate of cortical bones.

The analogous parameter of the carbonate-to-matrix ratio is an indication of total carbonate accumulation and it is related to bone turnover and remodeling activity [58]. Carbonate molecules play a major role in bone resorption; it is worth noting that lower concentration levels were reported for osteoporotic Eskimos [59]. Treated cortical samples had elevated carbonate content when compared with the control ones. For trabecular samples, this increase is evident only for group (ii). Biological apatite is a poorly crystalline form of carbonated apatite and the reduced crystallinity of the lattice is normally associated with mineral maturity [60]. Our osteoporotic samples of groups (i)–(iii) were of the same age and therefore we conclude that different supplementation does not interfere with the total carbonate accumulation (i.e., no significant differences between groups (i)–(iii)) although group (ii) constantly features higher values. Razzaque et al. found that vitamin D overload can accelerate the aging process in the aging-like phenotype of fibroblast growth factor 23 null mice [61]. Our results show that high vitamin D supplementation does not accelerate carbonate accumulation, as the carbonate content is constantly lower for group (i) than for groups (ii) and (iii). The lower

carbonate content for trabecular sections is in accordance with their high turnover rate, and the subsequent increased resorption leads to the above results of lower mineral-to-matrix ratios during mineralization.

3.3 Labile (non-apatitic) content

Band deconvolution of the well-defined $\text{CO}_3^{2-} \nu_2$ and $\text{PO}_4^{3-} \nu_4$ contours gives information about the type of mineral substitutions and especially of the non-apatitic (labile) components. Labile environments play a major role in bone mineral structural integrity due to their high reactivity; thus, they exhibit enhanced biological functionality and significance [62]. Bone mechanical strength as well as treatment response is heavily determined by acid phosphate content and its substitution into the bone mineral [63]. High mechanical strength is suggested to be associated with lower acid phosphate content in mice [64]. In addition, an increased concentration of the HPO_4^{2-} anion was noticed in ovariectomized osteoporotic monkeys [65] and in rabbits with inflammation induced osteoporosis [33].

In our case, labile acid phosphate (HPO_4^{2-}) content shows a highly significant decrease in the group (ii) bone samples, especially for the trabecular sections ($p < 0.001$). This result confirms our previous study [33] where it was demonstrated that these ions are inversely correlated with Ca^{2+} ions. There is a substantial replacement of PO_4^{3-} by HPO_4^{2-} in osteoporosis that creates an anionic vacancy to the hydroxyapatite lattice leading to the removal of calcium cations. Conversely, when mineral phosphate content is increasing (Fig. 2), excess calcium absorption compensates the lattice charge vacancy by simultaneous removal of non-apatitic acid phosphate ions. Groups (i) and (iii) (Fig. 2) show similar acid phosphate content that is higher than the control group. Group (iii) has a lower content than group (i) although this discrepancy is not significant. Newly deposited mineral features increased acid phosphate [66]. Thus, the lower acid phosphate content that is linked with the significant increase in the mineral-to-matrix ratio is an indication that the tissues of group (ii) reach high mineralization levels (formation of B-type apatite) similar to those of aged bone [12] due to high Ca intake. This is also supported by the elevated carbonate levels for this group.

The $\text{CO}_3^{2-} \nu_2$ band can be deconvoluted to three sub-bands at 879 cm^{-1} , 871 cm^{-1} , and 866 cm^{-1} (Fig. 1, inset) attributed to A-type, B-type, and labile carbonate content, respectively. Biological apatites are effectively B-type carbonate apatites with fractions of A-type and labile species. Comparison with the control group showed that the labile carbonate content of group (ii) remains practically unaffected while groups (i) and (iii) treated with excess vitamin D show increased concentrations in a constant statistically significant manner ($0.001 \leq p < 0.03$) regarding all bone sites except humerus samples.

Non-apatitic domains are strongly dependent on vitamin D deficiency. This is evident by a strong increase that reflects the modified rate of bone formation and resorption [67]. In our case, we observed a similar outcome where high doses of vitamin D (groups (i) and (iii)) significantly increase the labile carbonate content indicating new bone formation (Fig. 2). The increased concentration of the non-apatitic environments for high doses of vitamin D is also an indication of increased bone turnover. Although a major role of vitamin D is to suppress PTH and maintain normal bone metabolism, this effect has also been noticed in a recent study by Holvik et al. [68], where the authors suggested that the increased osteoclast activity may be due to a transient high turnover state reacting to the accelerating bone formation.

3.4 IMO model and vitamin D

The IMO protocol used in this study stimulates an acute phase response by granulomatous reactions and accumulation of inflammatory cells towards a significant decrease of the osteoblasts while osteoclasts and osteoclastic resorption are not affected [69]. Tumor necrosis factor- α (TNF- α) plays a major causal role in the IMO bone loss process [69, 70]. However, it is known that vitamin D acts as an immune system modulator enhancing the production of anti-inflammatory cytokines [71]. Specifically, 1,25(OH)D can significantly suppress TNF- α [72] while a possible direct link has been suggested between decreased levels of vitamin D and increased levels of the TNF- α cytokine [73]. Increased production of pro-inflammatory cytokines (like TNF- α and interleukin IL-6) is associated with increased bone resorption in diseases and malignancies like rheumatoid arthritis, periodontal disease, and osteolytic lesions of multiple myeloma [74]. Furthermore, ovariectomy in pre-menopausal women is linked to a significant increase in TNF- α secretion followed by increased bone resorption [75]. Other studies have further associated osteoporosis with inflammation [76, 77]. Consequently, osteoporosis is often considered as an inflammatory process and the IMO protocol effectively induces these symptoms by adversely altering bone metabolism.

The increased labile species in groups (i) and (iii) treated with high levels of vitamin D specifically imply the formation of new bone. Therefore, vitamin D supplementation suppresses resorption, restoring metabolic balance while calcium supplementation enhances the mineralization process. We also suggest that the increased mineralization index for group (i) is the result of the increased bone formation due to the anti-inflammatory properties of vitamin D. Thus, vitamin D is not only critical for circulating calcium concentrations levels but also for counterbalancing the effects of inflammation, while it is less significant for bone mineral content.

4 Conclusions

Osteoporosis is a common systemic skeletal disorder due to bone remodeling imbalance. Calcium- and vitamin D-deficient nutrition is critical for bone health. Calcium supplements typically suppress PTH secretion, slowing down bone resorption. Combined treatment with vitamin D has been shown to reduce fracture rates in elderly individuals where osteoporosis is a common disease. We utilized a protocol for osteoporosis involving induced inflammation supporting the concept that inflammation considerably contributes to the aetiopathogenesis of osteoporosis. Through vibrational spectroscopy we showed that a high dose of calcium supplementation (with or without vitamin D) can significantly increase bone mineralization under osteoporotic conditions. On the other hand, treatment with vitamin D only does not show a similar effect. We found increased carbonate content for the group with Ca supplementation accompanied by decreased labile concentrations indicative of stable apatitic environments. It is noteworthy that labile HPO_4^{2-} content is inversely correlated with Ca^{2+} ions in the hydroxyapatite lattice, giving the highest mineralization index for the group with the high Ca intake, similar to that of aged bone.

Vitamin D is strongly connected with bone health because it is vital for normal calcium and bone homeostasis. In this study, we found no clear evidence that it can independently promote mineralization even in high doses but only in combination with calcium. Vitamin D clearly plays a major role in the formation of the non-apatitic domains. Results show that a critical function of vitamin D under induced osteoporosis is to stimulate the formation of new bone through the suppression of the production of pro-inflammatory cytokines and to restore the metabolic balance of bone that was initially disturbed by induced inflammation.

Conflict of interest None.

References

1. Norman, A.W., Okamura, W.H., Bishop, J.E., Henry, H.L.: Update on biological actions of 1 alpha, 25(OH)(2)-vitamin D-3 (rapid effects) and 24R,25(OH)(2)-vitamin D-3. *Mol. Cell. Endocrinol.* **197**(1–2), 1–13 (2002)
2. Heaney, R.P.: The importance of calcium intake for lifelong skeletal health. *Calcified Tissue Int.* **70**(2), 70–73 (2002). doi:[10.1007/S00223-001-0032-3](https://doi.org/10.1007/S00223-001-0032-3)
3. Heaney, R.P.: Calcium, dairy products and osteoporosis. *J. Am. Coll. Nutr.* **19**(2), 83–99 (2000)
4. McLean, R.R., Qiao, N., Broe, K.E., Tucker, K.L., Casey, V., Cupples, L.A., Kiel, D.P., Hannan, M.T.: Dietary acid load is not associated with lower bone mineral density except in older men. *J. Nutr.* **141**(4), 588–594 (2011). doi:[10.3945/jn.110.135806](https://doi.org/10.3945/jn.110.135806)
5. Włodarek, D., Głabska, D., Kołota, A., Adamczyk, P., Czekajło, A., Grzeszczak, W., Drozdowska, B., Pluskiewicz, W.: Calcium intake and osteoporosis: the influence of calcium intake from dairy products on hip bone mineral density and fracture incidence—a population-based study in women over 55 years of age. *Public Health Nutr.* **17**(2), 383–389 (2014). doi:[10.1017/S1368980012005307](https://doi.org/10.1017/S1368980012005307)
6. Warensjö, E., Byberg, L., Melhus, H., Gedeberg, R., Mallmin, H., Wolk, A., Michaelsson, K.: Dietary calcium intake and risk of fracture and osteoporosis: prospective longitudinal cohort study. *Brit. Med. J.* **342**, (2011). doi: [10.1136/Bmj.D1473](https://doi.org/10.1136/Bmj.D1473)
7. Bischoff-Ferrari, H.A., Dawson-Hughes, B., Baron, J.A., Burckhardt, P., Li, R., Spiegelman, D., Specker, B., Orav, J.E., Wong, J.B., Staehelin, H.B., O'Reilly, E., Kiel, D.P., Willett, W.C.: Calcium intake and hip fracture risk in men and women: a meta-analysis of prospective cohort studies and randomized controlled trials. *Am. J. Clin. Nutr.* **86**(6), 1780–1790 (2007)
8. Xu, L.Z., McElduff, P., D'Este, C., Attia, J.: Does dietary calcium have a protective effect on bone fractures in women? A meta-analysis of observational studies. *Brit. J. Nutr.* **91**(4), 625–634 (2004). doi:[10.1079/Bjn20031085](https://doi.org/10.1079/Bjn20031085)
9. Lee, W.T.K., Leung, S.S.F., Leung, D.M.Y., Cheng, J.C.Y.: A follow-up study on the effects of calcium-supplement withdrawal and puberty on bone acquisition of children. *Am. J. Clin. Nutr.* **64**(1), 71–77 (1996)
10. Lee, W.T.K., Leung, S.S.F., Leung, D.M.Y., Wang, S.H., Xu, Y.C., Zeng, W.P., Cheng, J.C.Y.: Bone mineral acquisition in low calcium intake children following the withdrawal of calcium supplement. *Acta Paediatr.* **86**(6), 570–576 (1997). doi:[10.1111/j.1651-2227.1997.tb08936.x](https://doi.org/10.1111/j.1651-2227.1997.tb08936.x)
11. Varenna, M., Manara, M., Galli, L., Binelli, L., Zucchi, F., Sinigaglia, L.: The association between osteoporosis and hypertension: the role of a low dairy intake. *Calcified Tissue Int.* **93**(1), 86–92 (2013). doi:[10.1007/s00223-013-9731-9](https://doi.org/10.1007/s00223-013-9731-9)
12. Busse, B., Bale, H.A., Zimmermann, E.A., Panganiban, B., Barth, H.D., Carriero, A., Vettorazzi, E., Zustin, J., Hahn, M., Ager, J.W., Puschel, K., Amling, M., Ritchie, R.O.: Vitamin D deficiency induces early signs of aging in human bone, increasing the risk of fracture. *Sci. Transl. Med.* **5**(193), (2013). doi: [10.1126/scitranslmed.3006286](https://doi.org/10.1126/scitranslmed.3006286)
13. Heaney, R.P., Holick, M.F.: Why the IOM recommendations for vitamin D are deficient. *J. Bone Miner. Res.* **26**(3), 455–457 (2011). doi:[10.1002/Jbmr.328](https://doi.org/10.1002/Jbmr.328)
14. Need, A.G., O'Loughlin, P.D., Morris, H.A., Coates, P.S., Horowitz, M., Nordin, B.E.C.: Vitamin D metabolites and calcium absorption in severe vitamin D deficiency. *J. Bone Miner. Res.* **23**(11), 1859–1863 (2008). doi:[10.1359/Jbmr.080607](https://doi.org/10.1359/Jbmr.080607)
15. Heaney, R.P., Dowell, M.S., Hale, C.A., Bendich, A.: Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J. Am. Coll. Nutr.* **22**(2), 142–146 (2003)
16. Hansen, K.E., Jones, A.N., Lindstrom, M.J., Davis, L.A., Engelke, J.A., Shafer, M.M.: Vitamin D insufficiency: disease or no disease? *J. Bone Miner. Res.* **23**(7), 1052–1060 (2008). doi:[10.1359/Jbmr.080230](https://doi.org/10.1359/Jbmr.080230)
17. Binkley, N.: Vitamin D and osteoporosis-related fracture. *Arch. Biochem. Biophys.* **523**(1), 115–122 (2012). doi:[10.1016/j.abb.2012.02.004](https://doi.org/10.1016/j.abb.2012.02.004)
18. Looker, A.C., Mussolino, M.E.: Serum 25-hydroxyvitamin D and hip fracture risk in older US white adults. *J. Bone Miner. Res.* **23**(1), 143–150 (2008). doi:[10.1359/Jbmr.071003](https://doi.org/10.1359/Jbmr.071003)
19. Garnero, P., Munoz, F., Somay-Rendu, E., Delmas, P.D.: Associations of vitamin D status with bone mineral density, bone turnover, bone loss and fracture risk in healthy postmenopausal women. The OFELY study. *Bone* **40**(3), 716–722 (2007). doi:[10.1016/j.bone.2006.09.026](https://doi.org/10.1016/j.bone.2006.09.026)
20. Bouillon, R., Bischoff-Ferrari, H., Willett, W.: Vitamin D and health: perspectives from mice and man. *J. Bone Miner. Res.* **23**(7), 974–979 (2008). doi:[10.1359/Jbmr.080420](https://doi.org/10.1359/Jbmr.080420)

21. Noordam, R., de Craen, A.J.M., Pedram, P., Maier, A.B., Mooijaart, S.P., van Pelt, J., Feskens, E.J., Streppel, M.T., Slagboom, P.E., Westendorp, G.J., Beekman, M., van Heemst, D.: Levels of 25-hydroxyvitamin D in familial longevity: the Leiden Longevity Study. *Can. Med. Assoc. J.* **184**(18), E963–E968 (2012). doi:[10.1503/Cmaj.120233](https://doi.org/10.1503/Cmaj.120233)
22. Barone, A., Giusti, A., Pioli, G., Girasole, G., Razzano, M., Pizzonia, M., Palummeri, E., Bianchi, G.: Secondary hyperparathyroidism due to hypovitaminosis D affects bone mineral density response to alendronate in elderly women with osteoporosis: a randomized controlled trial. *J. Am. Geriatr. Soc.* **55**(5), 752–757 (2007). doi:[10.1111/j.1532-5415.2007.01161.x](https://doi.org/10.1111/j.1532-5415.2007.01161.x)
23. Ringe, J.D., Farahmand, P., Schacht, E., Rozeznal, A.: Superiority of a combined treatment of alendronate and alfacalcidol compared to the combination of alendronate and plain vitamin D or alfacalcidol alone in established postmenopausal or male osteoporosis (AAC-Trial). *Rheumatol. Int.* **27**(5), 425–434 (2007). doi:[10.1007/s00296-006-0288-z](https://doi.org/10.1007/s00296-006-0288-z)
24. Mastaglia, S.R., Pellegrini, G.G., Mandalunis, P.M., Chaves, M.M.G., Friedman, S.M., Zeni, S.N.: Vitamin D insufficiency reduces the protective effect of bisphosphonate on ovariectomy-induced bone loss in rats. *Bone* **39**(4), 837–844 (2006). doi:[10.1016/j.bone.2006.04.015](https://doi.org/10.1016/j.bone.2006.04.015)
25. Klibanski, A., Adams-Campbell, L., Bassford, T., Blair, S.N., Boden, S.D., Dickersin, K., Gifford, D.R., Glaspe, L., Goldring, S.R., Hruska, K., Johnson, S.R., McCauley, L.K., Russell, W.E.: Osteoporosis prevention, diagnosis, and therapy. *J. Am. Med. Assoc.* **285**(6), 785–795 (2001)
26. Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., Kovacs, C.S., Mayne, S.T., Rosen, C.J., Shapses, S.A.: The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocr. Metab.* **96**(1), 53–58 (2011). doi:[10.1210/Jc.2010-2704](https://doi.org/10.1210/Jc.2010-2704)
27. Dietary Reference Intakes for Calcium and Vitamin D. The National Academies Press. OpenBook http://www.nap.edu/catalog.php?record_id=13050 (2011). Accessed 19 Jan 2014
28. Joo, N.S., Dawson-Hughes, B., Kim, Y.S., Oh, K., Yeum, K.J.: Impact of calcium and vitamin D insufficiencies on serum parathyroid hormone and bone mineral density: analysis of the fourth and fifth Korea National Health and Nutrition Examination Survey (KNHANES IV-3, 2009 and KNHANES V-1, 2010). *J. Bone Miner. Res.* **28**(4), 764–770 (2013). doi:[10.1002/Jbmr.179](https://doi.org/10.1002/Jbmr.179)
29. Heaney, R.P., Weaver, C.M.: Calcium and vitamin D. *Endocrin. Metab. Clin.* **32**(1), 181 (2003). doi:[10.1016/S0889-8529\(02\)00063-4](https://doi.org/10.1016/S0889-8529(02)00063-4)
30. Nieves, J.W., Barrett-Connor, E., Siris, E.S., Zion, M., Barlas, S., Chen, Y.T.: Calcium and vitamin D intake influence bone mass, but not short-term fracture risk, in Caucasian postmenopausal women from the National Osteoporosis Risk Assessment (NORA) study. *Osteoporosis Int.* **19**(5), 673–679 (2008). doi:[10.1007/s00198-007-0501-2](https://doi.org/10.1007/s00198-007-0501-2)
31. Prestwood, K.M., Pannullo, A.M., Kenny, A.M., Pilbeam, C.C., Raisz, L.G.: The effect of a short course of calcium and vitamin D on bone turnover in older women. *Osteoporosis Int.* **6**(4), 314–319 (1996). doi:[10.1007/Bf01623391](https://doi.org/10.1007/Bf01623391)
32. Kourkoulis, N., Balatsoukas, I., Tzaphlidou, M.: Ca/P concentration ratio at different sites of normal and osteoporotic rabbit bones evaluated by Auger and energy-dispersive X-ray spectroscopy. *J. Biol. Phys.* **38**(2), 279–291 (2012). doi:[10.1007/s10867-011-9247-3](https://doi.org/10.1007/s10867-011-9247-3)
33. Kourkoulis, N., Lani, A., Tzaphlidou, M.: Infrared spectroscopic assessment of the inflammation-mediated osteoporosis (IMO) model applied to rabbit bone. *J. Biol. Phys.* **38**(4), 623–635 (2012). doi:[10.1007/s10867-012-9276-6](https://doi.org/10.1007/s10867-012-9276-6)
34. Bonjour, J.P., Ammann, P., Rizzoli, R.: Importance of preclinical studies in the development of drugs for treatment of osteoporosis: a review related to the 1998 WHO guidelines. *Osteoporosis Int.* **9**, 379–393 (1999)
35. Duque, G., Watanabe, K. (eds.): *Osteoporosis Research: Animal Models*. Springer (2011)
36. Turner, A.S.: Animal models of osteoporosis—necessity and limitations. *Eur. Cells Mater.* **1**, 66–81 (2001)
37. Jee, W.S.S., Yao, W.: Overview: animal models of osteopenia and osteoporosis. *J. Musculoskel. Neuron. Interact.* **1**(3), 193–207 (2001)
38. Castañeda, S., Calvo, E., Largo, R., González-González, R., de la Piedra, C., Díaz-Curiel, M., Herrero-Beaumont, G.: Characterization of a new experimental model of osteoporosis in rabbits. *J. Bone Miner. Metab.* **26**(1), 53–59 (2008). doi:[10.1007/s00774-007-0797-1](https://doi.org/10.1007/s00774-007-0797-1)
39. Baofeng, L., Zhi, Y., Bei, C., Guolin, M., Qingshui, Y., Jian, L.: Characterization of a rabbit osteoporosis model induced by ovariectomy and glucocorticoid. *Acta Orthop.* **81**(3), 396–401 (2010). doi:[10.3109/17453674.2010.483986](https://doi.org/10.3109/17453674.2010.483986)
40. Mashiba, T., Burr, D.B., Turner, C.H., Sato, M., Cain, R.L., Hock, J.M.: Effects of human parathyroid hormone (1–34), LY333334, on bone mass, remodeling, and mechanical properties of cortical bone during the first remodeling cycle in rabbits. *Bone* **28**(5), 538–547 (2001). doi:[10.1016/S8756-3282\(01\)00433-1](https://doi.org/10.1016/S8756-3282(01)00433-1)
41. Nutrient Requirements of Rabbits. National Research Council. Washington, DC: National Academy of Sciences (1977)

42. Bolland, M., Avenell, A., Baron, J.A., Grey, A., MacLennan, G.S., Gamble, G.D., Reid, I.R.: Effect of calcium supplements on the risk of myocardial infarction and cardiovascular events: a meta-analysis. *Osteoporosis Int.* **21**, 21–21 (2010)
43. Jackson, R.D., LaCroix, A.Z., Gass, M., Wallace, R.B., Robbins, J., Lewis, C.E., Bassford, T., Beresford, S.A.A., Black, H.R., Blanchette, P., Bonds, D.E., Brunner, R.L., Brzyski, R.G., Caan, B., Cauley, J.A., Chlebowski, R.T., Cummings, S.R., Granek, I., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Johnson, K.C., Judd, H., Kotchen, J.M., Kuller, L.H., Langer, R.D., Lasser, N.L., Limacher, M.C., Ludlam, S., Manson, J.E., Margolis, K.L., McGowan, J., Ockene, J.K., O'Sullivan, M.J., Phillips, L., Prentice, R.L., Sarto, G.E., Stefanick, M.L., Van Horn, L., Wactawski-Wende, J., Whitlock, E., Anderson, G.L., Assaf, A.R., Barad, D., Investigat, W.H.I.: Calcium plus vitamin D supplementation and the risk of fractures. *New Engl. J. Med.* **354**(7), 669–683 (2006). doi:[10.1056/Nejmoea055218](https://doi.org/10.1056/Nejmoea055218)
44. Hsia, J., Heiss, G., Ren, H., Allison, M., Dolan, N.C., Greenland, P., Heckbert, S.R., Johnson, K.C., Manson, J.E., Sidney, S., Trevisan, M.: Calcium/vitamin D supplementation and cardiovascular events. *Circulation* **115**(7), 846–854 (2007). doi:[10.1161/Circulationaha.106.673491](https://doi.org/10.1161/Circulationaha.106.673491)
45. Bolland, M.J., Grey, A., Avenell, A., Gamble, G.D., Reid, I.R.: Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *Brit. Med. J.* **342** (2011). doi: [10.1136/Bmj.D2040](https://doi.org/10.1136/Bmj.D2040)
46. Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M., Vasan, R.S.: Vitamin D deficiency and risk of cardiovascular disease. *Circulation* **117**(4), 503–511 (2008). doi:[10.1161/Circulationaha.107.706127](https://doi.org/10.1161/Circulationaha.107.706127)
47. Hanley, D.A., Cranney, A., Jones, G., Whiting, S.J., Leslie, W.D., Cole, D.E.C., Atkinson, S.A., Josse, R.G., Feldman, S., Kline, G.A., Rosen, C., Osteoporosis, S.A.C.: Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. *Can. Med. Assoc. J.* **182**(12), E610–E618 (2010). doi: [10.1503/Cmaj.080663](https://doi.org/10.1503/Cmaj.080663)
48. Kourkoulis, N., Tzaphlidou, M.: Spectroscopic assessment of normal cortical bone: differences in relation to bone site and sex. *Sci. World J.* **10**, 402–412 (2010). doi:[10.1100/tsw.2010.43](https://doi.org/10.1100/tsw.2010.43)
49. Gu, C., Katti, D.R., Katti, K.S.: Photoacoustic FTIR spectroscopic study of undisturbed human cortical bone. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **103**, 25–37 (2013). doi:[10.1016/j.saa.2012.10.062](https://doi.org/10.1016/j.saa.2012.10.062)
50. Paschalis, E.P., Mendelsohn, R., Boskey, A.L.: Infrared assessment of bone quality: a review. *Clin. Orthop. Relat. Res.* **469**(8), 2170–2178 (2011). doi:[10.1007/s11999-010-1751-4](https://doi.org/10.1007/s11999-010-1751-4)
51. Turner, C.H., Hinkley, W.R., Wilson, M.E., Zhang, W., Dunipace, A.J.: Combined effects of diets with reduced calcium and phosphate and increased fluoride intake on vertebral bone strength and histology in rats. *Calcified Tissue Int.* **69**(1), 51–57 (2001). doi:[10.1007/s002230010033](https://doi.org/10.1007/s002230010033)
52. Boivin, G.Y., Chavassieux, P.M., Santora, A.C., Yates, J., Meunier, P.J.: Alendronate increases bone strength by increasing the mean degree of mineralization of bone tissue in osteoporotic women. *Bone* **27**(5), 687–694 (2000). doi:[10.1016/S8756-3282\(00\)00376-8](https://doi.org/10.1016/S8756-3282(00)00376-8)
53. Gadeleta, S.J., Boskey, A.L., Paschalis, E., Carlson, C., Menschik, F., Baldini, T., Peterson, M., Rinnac, C.M.: A physical, chemical, and mechanical study of lumbar vertebrae from normal, ovariectomized, and nandrolone decanoate-treated cynomolgus monkeys (*Macaca fascicularis*). *Bone* **27**(4), 541–550 (2000). doi:[10.1016/S8756-3282\(00\)00362-8](https://doi.org/10.1016/S8756-3282(00)00362-8)
54. Reid, I.R., Bolland, M.J., Grey, A.: Effects of vitamin D supplements on bone mineral density: a systematic review and meta-analysis. *Lancet* **338**(9912), 146–155 (2014). doi:[10.1016/S0140-6736\(13\)61647-5](https://doi.org/10.1016/S0140-6736(13)61647-5)
55. Lieben, L., Masuyama, R., Torrekens, S., Van Looveren, R., Schrooten, J., Baatsen, P., Lafage-Proust, M.H., Dresselaers, T., Feng, J.Q., Bonewald, L.F., Meyer, M.B., Pike, J.W., Bouillon, R., Carmeliet, G.: Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. *J. Clin. Invest.* **122**(5), 1803–1815 (2012). doi:[10.1172/Jci45890](https://doi.org/10.1172/Jci45890)
56. Holick, M.F.: Evolution, biologic functions, and recommended dietary allowances for vitamin D. In: Holick, M.F. (ed.) *Vitamin D: physiology, molecular biology, and clinical applications*, pp. 1–16. Humana Press, Totowa (1999)
57. Balsan, S., Garabedian, M., Larchet, M., Gorski, A.M., Coumot, G., Tau, C., Bourdeau, A., Silve, C., Ricour, C.: Long-term nocturnal calcium infusions can cure rickets and promote normal mineralization in hereditary resistance to 1,25-dihydroxyvitamin-D. *J. Clin. Invest.* **77**(5), 1661–1667 (1986). doi:[10.1172/Jci112483](https://doi.org/10.1172/Jci112483)
58. Isaksson, H., Turunen, M.J., Rieppo, L., Saarakkala, S., Tamminen, I.S., Rieppo, J., Kroger, H., Jurvelin, J.S.: Infrared spectroscopy indicates altered bone turnover and remodeling activity in renal osteodystrophy. *J. Bone Miner. Res.* **25**(6), 1360–1366 (2010). doi:[10.1002/Jbmr.10](https://doi.org/10.1002/Jbmr.10)
59. Thompson, D.D., Posner, A.S., Laughlin, W.S., Blumenthal, N.C.: Comparison of bone apatite in osteoporotic and normal Eskimos. *Calcified Tissue Int.* **35**(3), 392–393 (1983). doi:[10.1007/Bf02405064](https://doi.org/10.1007/Bf02405064)
60. Farlay, D., Panczer, G., Rey, C., Delmas, P.D., Boivin, G.: Mineral maturity and crystallinity index are distinct characteristics of bone mineral. *J. Bone Miner. Metab.* **28**(4), 433–445 (2010). doi:[10.1007/s00774-009-0146-7](https://doi.org/10.1007/s00774-009-0146-7)

61. Razzaque, M.S., Sitara, D., Taguchi, T., St-Arnaud, R., Lanske, B.: Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. *FASEB J.* **20**(1), 720–722 (2006). doi:[10.1096/fj.05-5432fje](https://doi.org/10.1096/fj.05-5432fje)
62. Rey, C., Collins, B., Goehl, T., Dickson, I.R., Glimcher, M.J.: The carbonate environment in bone-mineral—a resolution-enhanced fourier-transform infrared-spectroscopy study. *Calcified Tissue Int.* **45**(3), 157–164 (1989). doi:[10.1007/Bf02556059](https://doi.org/10.1007/Bf02556059)
63. Spevak, L., Flach, C., Hunter, T., Mendelsohn, R., Boskey, A.: Fourier transform infrared spectroscopic imaging parameters describing acid phosphate substitution in biologic hydroxyapatite. *Calcified Tissue Int.* **92**(5), 418–428 (2013). doi:[10.1007/s00223-013-9695-9](https://doi.org/10.1007/s00223-013-9695-9)
64. Courtland, H.W., Nasser, P., Goldstone, A.B., Spevak, L., Boskey, A.L., Jepsen, K.J.: Fourier transform infrared imaging microspectroscopy and tissue-level mechanical testing reveal intraspecies variation in mouse bone mineral and matrix composition. *Calcified Tissue Int.* **83**(5), 342–353 (2008). doi:[10.1007/s00223-008-9176-8](https://doi.org/10.1007/s00223-008-9176-8)
65. Huang, R.Y., Miller, L.M., Carlson, C.S., Chance, M.R.: Characterization of bone mineral composition in the proximal tibia of cynomolgus monkeys: effect of ovariectomy and nandrolone decanoate treatment. *Bone* **30**(3), 492–497 (2002). doi:[10.1016/S8756-3282\(01\)00691-3](https://doi.org/10.1016/S8756-3282(01)00691-3)
66. Faibish, D., Gomes, A., Boivin, G., Binderman, I., Boskey, A.: Infrared imaging of calcified tissue in bone biopsies from adults with osteomalacia. *Bone* **36**(1), 6–12 (2005). doi:[10.1016/j.bone.2004.08.019](https://doi.org/10.1016/j.bone.2004.08.019)
67. Rey, C., Lian, J., Grynaps, M., Shapiro, F., Zylberberg, L., Glimcher, M.J.: Non-apatitic environments in bone-mineral—Ft-Ir detection, biological properties and changes in several disease states. *Connect. Tissue Res.* **21**(1–4), 597–603 (1989)
68. Holvik, K., Madar, A.A., Meyer, H.E., Lofthus, C.M., Stene, L.C.: Changes in the vitamin D endocrine system and bone turnover after oral vitamin D3 supplementation in healthy adults: results of a randomised trial. *BMC Endocr Disord* **12** (2012). doi: [10.1186/1472-6823-12-7](https://doi.org/10.1186/1472-6823-12-7)
69. Armour, K.J., Armour, K.E.: Methods in molecular medicine, vol. 80: Bone research protocols: inflammation-induced osteoporosis, The IMO Model. Humana Press Inc, Totowa (2003)
70. Vukicević, S., Marusić, A., Stavljenić, A., Cesnjaj, M., Ivanković, D.: The role of tumor-necrosis-factor-alpha in the generation of acute-phase response and bone loss in rats with talc granulomatosis. *Lab. Invest.* **70**(3), 386–391 (1994)
71. Cantorna, M.T., Woodward, W.D., Hayes, C.E., DeLuca, H.F.: 1,25-Dihydroxyvitamin D3 Is a positive regulator for the two anti-encephalitogenic cytokines TGF- β 1 and IL-4. *J. Immunol.* **160**(11), 5314–5319 (1998)
72. Kuo, Y.-T., Kuo, C.-H., Lam, K.-P., Chu, Y.-T., Wang, W.-L., Huang, C.-H., Hung, C.-H.: Effects of vitamin D3 on expression of tumor necrosis factor- α and chemokines by monocytes. *J. Food Sci.* **75**(6), H200–H204 (2010). doi:[10.1111/j.1750-3841.2010.01704.x](https://doi.org/10.1111/j.1750-3841.2010.01704.x)
73. Willis, K.S., Smith, D.T., Broughton, K.S., Larson-Meyer, D.E.: Vitamin D status and biomarkers of inflammation in runners. *Open Access J. Sports Med.* **3**, 35–42 (2012). doi:[10.2147/OAJSM.S31022](https://doi.org/10.2147/OAJSM.S31022)
74. Mundy, G.R.: Osteoporosis and inflammation. *Nutr. Rev.* **65**, S147–S151 (2007). doi:[10.1111/j.1753-4887.2007.tb00353.x](https://doi.org/10.1111/j.1753-4887.2007.tb00353.x)
75. Ammann, P., Rizzoli, R., Bonjour, J.P., Bourrin, S., Meyer, J.M., Vassalli, P., Garcia, I.: Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. *J. Clin. Invest.* **99**(7), 1699–1703 (1997). doi:[10.1172/JCI119333](https://doi.org/10.1172/JCI119333)
76. Yun, A.J., Lee, P.Y.: Maldaptation of the link between inflammation and bone turnover may be a key determinant of osteoporosis. *Med. Hypotheses* **63**(3), 532–537 (2004)
77. Lerner, U.H.: Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. *J. Dent. Res.* **85**(7), 596–607 (2006). doi:[10.1177/154405910608500704](https://doi.org/10.1177/154405910608500704)