**REVIEW**

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# Selected aspects of osteoporosis prevention

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**Abstract:** Osteporosis affects a large percentage of postmenopausal women and leads to serious complications such as femur neck fracture. The standard of osteoporosis diagnosis is dual-energy X-ray absorptiometry (DXA) which evaluates bone mineral content and after dividing it by measurement area estimates bone mineral density. There are also biochemical indicators evaluated in human serum or urine such as calcium urine concentration, piridinoline, deoksypiridinoline (DPR), and collagene fragments, such as cross-linked N-telopeptide (NTX) and cross-linked C-telopeptide (CTX), which may be useful for determining the activity of bone formation and resorption processes. They are frequently used for scientific purposes, but not in general medical practice. Osteoporosis risk factors include: age over 70 years, low weight (below 40 kg) or body mass index (BMI) below 20, weight loss of more than 10% of total body mass, low physical activity, chronic treatment with glucocorticoids or anti-epileptic drugs, anorexia nervosa, type 1 diabetes mellitus, past gastrectomy, and hyperparathyroidismus. There are also some other conditions which may affect bone metabolism which include: female sex, smoking, cases of fractures in family, a history of ovariectomy, early menopause (before the age of 45), low calcium consumption, hyperthyroidismus, rheumatoid arthritis, chronic alcoholism, excessive vitamin A intake and cola drinks. Also, adequate intake of proteins and macro- and microelements may affect bone metabolism. Some epidemiological studies suggest that higher consumption of soy bean based foods in Asian populations compared to Western populations may be responsible for the low incidence of postmenopausal osteoporosis in Asian women. Soy beans are a rich source of isoflavones, mainly genistein and daidzein, which have both weak estrogenic and antiestrogenic effects. Isoflavones bind to estrogen receptors in body tissues such as uterine or bones, and some data suggest that they may stimulate osteoblastic activity and bone formation, and also inhibit osteoclastic bone resorption. In this way, they may prevent postmenopausal bone loss. The protective effects of other natural substances such as rhizome of *Anemarrhena aspheloides* or aqueus extract of black tea have also been investigated. This article presents a review of food contents and lifestyle factors which influence bone metabolism.

**Key words:** osteoporosis, bones, prevention

## INTRODUCTION

As a result of an increase in the average life span and popularization of the western lifestyle among the population there is a rise in the incidence of chronic diseases with a risk of occurrence that varies with a patient's age. Such diseases include circulatory system disorders, type 2 diabetes, dementia and osteoporosis.

According to the definition of World Health Organization (WHO) osteoporosis is a systemic skeletal disease, characterized by low bone mass and disorders in bone microarchitecture which lead to increased risk of fractures. In osteoporosis diagnosis, a commonly used parameter is the T-score, described as a standard deviation from peak bone mass – bone mass of young, healthy Caucasian women achieved between 20-29 years of age, and the Z-score which compares bone mass value of examined woman with the mean bone mass value of women at the same age. Osteoporosis is defined by a T-score value decreased below -2.5. Another definition from the National Osteoporosis Foundation and National Institutes of Health describes it as a skeletal disease, characterized by lowered bone resistance, which leads to increased fracture risk [1].

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**Diagnosis of osteoporosis.** Osteoporosis is characterized by lowered bone mineral density (BMD). At present there are no methods used in common medical practice which can measure microstructure or bone resistance. The standard diagnosis of osteoporosis is based on dual-energy X-ray absorptiometry (DXA) measurement. This estimates the value of bone mineral content (BMC), and dividing it by the measurement area gives the bone mineral density (BMD) value. BMD lowered to -2.5 SD (standard deviation in relation to peak bone mass) or below is described as osteoporosis. However, it has to be interpreted in connection with other individual patients' data. The most common sites for evaluating BMD are the lumbar vertebrae, femoral neck, femoral trochanter, Ward's triangle and total proximal femur epiphysis, as well as one-third of the distal part of the radius. BMD value obtained from different sites may differ even in the same person. The measurement of femur BMD is known as the most reliable method of total fracture risk evaluation and is absolutely essential in making the decision of treatment initiation [1].

Standard radiograms are also useful in the diagnosis of osteoporosis. They can reveal typical vertebrae deformations which are the result of compressive fractures. Quantative ultrasound method enables risk fracture evaluation by the examination of peripheral bones. It measures such parameters as the speed of sound (SOS) and broad-band ultrasound attenuation (BUA). These parameters are used to calculate the stiffness parameter and its value can be given as a T-score.

Opinions on its effectiveness in osteoporosis diagnosis are divided, but it seems to be a useful method in fracture risk evaluation. Quantative computed tomography is occasionally used for scientific purposes, but it is not used in common medical practice. This gives a possibility to distinguish volumetric bone mineral density between trabecular and cortical bone tissue [2, 3].

Biochemical indicators evaluated in human serum or urine may be used to determine the activity of bone formation and resorption processes. The markers of bone resorption include calcium urine concentration, pirydinoline, deoksypiridinoline (DPR), and collagene fragments such as cross-linked N-telopeptide (NTX) and cross-linked C-telopeptide (CTX). Bone formation markers include: bone-specific alkaline phosphatase (BAP), osteocalcin, procollagen type I aminoterminal peptide (PINP) and procollagen type I carboxyterminal peptide (PICP) [4]. These are widely used for scientific purposes, but not in general medical practice.

**Osteoporosis risk factors and prevention.** Several conditions are considered to be osteoporosis risk factors. High risk factors include, for example: age over 70 years, low weight (below 40 kg) or body mass index (BMI) below 20, weight loss of more than 10% of total body mass, low physical activity, chronic treatment with glucocorticoids or anti-epileptic drugs, anorexia nervosa, type 1 diabetes mellitus, past gastrectomy, and hyperparathyroidismus. Other conditions which may affect bone metabolism include: female sex, smoking, cases of fractures in family, a history of ovariectomy, early menopause (before the age of 45), low calcium consumption, hyperthyroidismus, rheumatoid arthritis, chronic alcoholism, excessive vitamin A intake and cola drinks [3]. Adequate intake of vitamins D, C and K, proteins, soy isoflavones, macro- and microelements such as calcium, phosphorus, magnesium, zinc, fluoride, copper, iron and selenium, as well as physical activity have a beneficial influence on bone tissue in terms of osteoporosis prevention.

After the age of 30, both men and women lose about 0.3-0.8% of their bone mass per year. This is due to alterations in osteoblast functions, lowered activity of 1 -hydroxylase of 25-hydroxyvitamin $D_3$, and decreased intestinal sensitivity to 1,25-dihydroxyvitamin $D_3$. After menopause, more rapid bone mass decrease occurs, particularly in the first few years. In this period of life, a woman can lose 1.2-6% of total bone mass per year. Trabecular bone is more active metabolically, and is therefore much more sensitive to resorptive factors than corticall bone. Trabecular bone density decreases by about 5-8% per year, whereas cortical bone density decreases by only about 1-3% per year [5]. Preventive actions of osteoporosis are even more important, because due to epidemiological data the life-long risk of femoral neck fracture is 46.4% for women aged 50 and 22.4% for men at the same age. During the first year after having their femoral neck fractured, 20% of women and 30% of men die due to complications, and 50% of those who remain alive are physically disabled [6]. There are many studies which evaluate the influence of several factors such as physical activity, nutrition and some other natural substances contained in plants on the prevention of postmenopausal osteoporosis.

Bone, especially trabecular bone, is a dynamic tissue and its mass and architecture depend on the balance between resorption and formation. It has been proved that mechanical stresses acting during physical activity lead to the increase of bone mass. This effect may be mediated through insulin-like growth factors released by multiple tissues and can act both in an endocrine and autocrine/paracrine way [7]. Osteoblasts produce osteoid, which is composed mainly of type I collagen, and responsible for the mineralization of osteoid matrix. Osteoclasts, located in mineralized bone tissue, are able to convert data about mechanical strains into signals which affect osteoblastic activity [8]. Immobilisation decreases osteoblast activity and increases resorptive processes. Osteoclasts release hydrogen ions and dissolve mineralized bone matrix. After menopause, the lack of estrogens leads to an increase of bone turnover and osteoclastic resorption, and results in significant loss of bone mass. Additionally, there is much evidence that estrogens may increase intestinal absorption of calcium by direct action on estrogen receptors in the intestine. These receptors are also present in kidney tubules and regulate calcium reabsorption. It has been proved that after menopause intestinal absorption and kidney reabsorption of calcium is significantly decreased, which leads to negative calcium balance [10]. Gala et al. investigated the short- and long-term effects of physical activity and calcium administration on bone mineral density in ovariectomized rats [7]. As a result, after 12 weeks of treatment, femoral BMD in exercise-alone group and exercise-plus-Ca-supplementation group increased significantly compared to control ovariectomized rats without any treatment (12.4% and 10.3% versus 21.4%, respectively). Despite this, femoral BMD was still significantly lower than the control sham group. After 27 weeks of treatment, only the group with combined exercise and Ca supplementation showed a significant increase of femoral BMD compared with control ovariectomized rats (20.8 versus 28.0% bone loss). In a similar way to femoral BMD changes, exercises combined with Ca induced the strongest effect on lumbar BMD [7].

Some epidemiological studies suggest that the low incidence of postmenopausal osteoporosis in Asian women compared to Western women may depend on their higher consumption of soy bean based foods. The positive effect of soy beans on BMD and mechanical bone strength may be associated with their amino acid composition. Soy beans are also a rich source of isoflavones, mainly genistein and daidzein, which have both weak estrogenic and antiestrogenic effects and have structural similarities to tamoxifen [10]. Isoflavones bind to estrogen receptors in body tissues such as uterine and bones. Data suggest that they may stimulate osteoblastic activity and bone formation and inhibit osteoclastic bone resorption. Consequently, they prevent bone loss after menopause. It has been noted that the consumption of phytoestrogens in soy foods may be the reason for relatively low rates of breast, prostate and colon cancer, and also lowered the incidence of cardiovascular diseases in the Japanese and Chinese populations [11].

One study revealed that both isoflavone and 17β-estradiol exhibited similar bone-sparing ability in ovariectomised rats [10]. The effects on metabolic bone turnover markers were not the same; estrogens but not isoflavones suppressed the increase of AP and tartrate–resistant acid phosphatase (TRAP) activity in ovariectomized rats. The uterine weight was greater in ovariectomized rats treated with 17β-estradiol than both in the group treated with isoflavone and ovariectomized control group [10]. Another study revealed that treating ovariectomized rats with isoflavones led to the increase of trabecular thickeness and number, and lowered trabecular separation. This effect has not been proved for soya proteins

[12]. Combined intervention of moderate exercise (running) and isoflavones prevented bone loss in ovariectomized mice. Bone mineral density of the whole body, femur or lumbar spine was reduced by ovariectomy, and the bone loss was partially prevented by exercise or isoflavone alone. However, the combined intervention (exercise + isoflavones) restored the bone mass to the level of the non-ovariectomized control group [13]. Combined intervention of dietary soy bean proteins and swimming training revealed that soy bean protein supplementation alone increased bone calcium content, decreased the level of osteocalcin in plasma, but had no effect on calcium balance and absorption. Swimming training alone increased plasma and bone calcium content as well as calcium absorption and balance, but did not affect either plasma osteocalcin values or urinary deoxypiridinoline excretion. Combined intervention elevated plasma and bone calcium content, but had no effect on calcium absorption or bone resorption markers. The authors suggest that combined intervention does not have a synergistic effect on calcium metabolism and bone resorption markers [14]. In another study, ovariectomised rats were treated with isoflavone-supplemented soy yoghurt, resistive physical exercise, or both. As a result, BMD values in femur and tibia and AP level increased in all treated groups [11].

Experimental data suggest that isoflavones with calcium supplementation protect against loss of bone mass and strength after ovariectomy more effectively than isoflavones alone. Femur and vertebra BMD values were higher in rats treated with combined intervention than in the groups treated with isoflavones or exercise alone [15]. Another study did not confirm bone protective role of soy in ovariectomized rats; however, in this study, rats were given soy- or casein-based diets with or without isoflavones for 8 weeks, and a reference group was treated with 17β-estradiol. As a result, isoflavones given separately or with soy protein did not prevent the loss of trabecular bone. Isoflavones added to estrogen therapy had no additional preventive effects on bone loss. However, estrogen therapy prevented bone loss from both trabecular and cortical bone. Neither soy protein nor isoflavones had an influence on calcium absorption. Soy protein significantly decreased urinary calcium loss, which has been explained by researchers as the result of a lower level of sulfur-containing amino acids in soy protein [9].

The biological effects of genistein and daidzein have not yet been fully investigated. Genistein probably inhibits osteoclastic activity and protein tyrosine kinases. It may also have a stimulatory effect on bone formation and mineralization [9]. A study using a radioactive cDNA microarray also suggested that genistein modulates bone metabolism-related gene expression, including bone matrix proteins, calciotropic receptor, cytokines and growth factors [16]. Another study revealed preventive effects of genistein treatment and resistant exercise in ovariectomised rats. Genistein and exercise alone equally elevated BMD of ovariectomized rats by 5%, but combined therapy recovered their lowered BMD by 8% [17].

It has been reported that daidzein inhibits mitochondrial aldehyde dehydrogenase and also has an anabolic influence on bone tissue cultures. Daidzein is metabolized from its natural form, daidzin, by β-glucosidase enzymes produced by bacteria in the gastrointestinal tract. If there is a sufficient number of bacteria in the bowel, daidzein is futher converted to equol, which has a higher affinity to estrogen receptor than daidzein. In another study, daidzein alone did not prevent trabecular bone loss in ovariectomised mice. However, combined treatment with daidzein and calcium had positive effects on cortical and trabecular bone [18]. In yet another study, ovariectomized rats were given genistein or equol, or its precursor, daizein, and provided with short-chain fructooligosaccharydes modulating intestinal flora or live Lactobacillus casei. As a result of the experiment, all three substances provided bone loss protecting effects. Supplementation with short-chain fructooligosaccharides and Lactobacillus additionally raised the efficiency of daidzein [19].

The role of another isoflavone, glycitein, which accounts for about 5-10% of isoflavones in soy food, has not yet been determined. Some data suggest that it also has a weak estrogenic activity, but its effect on bone metabolism requires further investigation [9].

The protective effects of other natural substances have also been investigated. Rhizome of *Anemarrhena asphodeloides* – a popular plant in China – containing steroidal saponins, was one of them. The study revealed that the treatment of ovariectomized rats with this plant resulted in the suppression of BMD decrease. Also it corrected the lowered level of calcium and estradiol in plasma, prevented the decrease of trabecular thickeness and the increase of trabecular separation in ovariectomized rats [20]. Another study examined the effect of *Epimedii sagittatum* – a plant used in Chinese herbal medicine for over 2,000 years – on bone metabolism in ovariectomized rats. As a result, it suppressed the decrease of BMD, corrected lowered concentrations of calcium and estradiol in serum, and increased AP activity. It also prevented the increase of trabecular separation, but did not influence trabecular thickeness or number [21]. Aqueous extract of black tea *(Camelia sinensis*) may also have an antiosteoporotic effect. Administered to bilaterally oophorectomized rats, it led to a significant increase of serum estradiol and tartrate-resistant acid phosphatase activity and decrease urinary hydroxyproline level. Except for femur, treatment with black tea extract restored the density of the other investigated bones (eighth thoracic rib, eighth thoracic vertebra and fourth lumbar vertebra) [22].

Nitric oxide (NO) is also supposed to have an influence on bone metabolism, probably through the stimulation of osteoblastic activity and restraining of osteoclastic resorption. Ovariectomized rats treated with transdermal nitroglycerin or 17-β-estradiol maintained the same BMD level as non-ovariectomized controls [23].

Osteoporosis is a multi-factorial disease. Up to 80% of bone strength may be determined genetically. Other determinants are nutritional and lifestyle factors, as well as hormonal balance and physiological conditions: age, height, weight, muscle and fat content. Some of these factors, for example genotype, cannot be affected, but the others, such as nutrition or physical activity can be modified. Almost 80-90% of bone mineral content is composed of calcium and phosphorus. Protein intake also plays an important role in bone formation. Collagen formation in organic bone matrix enables further mineralization. Protein also takes part in the regulation of calcium absorption. Other minerals, such as magnesium, zinc, fluoride, iron, copper, selenium and vitamins D, A, C, K and folate are also involved in bone formation and resorption processes [24].

The cross-sectional study in the group of 136 healthy Caucasian women after menopause investigated the influence of various nutrients and energy intake on BMD. It revealed a strong influence of calcium, energy and protein intake on

BMD of several bones. Significant relation to BMD has been also revealed for vitamin C, zinc and magnesium [24].

A low BMD is known to be an osteoporosis risk factor. There are several studies concerning the possible effects of lifestyle osteoporosis risk factors on people with different BMI categories. One of the studies investigated a group of 1,222 women aged 70-73 years with different BMI values. A self-obtained questionnaire was used to acquire data about past and present occupational and leisure time activities, calcium and alcohol intake, smoking and medical history. A reducing BMD influence of lifestyle factors such as low physical activity at work, low habitual exercise at the ages of 30 and 50 years, and at present, drinking of 5 or more cups of coffee a day was significant in the group of lean women. These factors did not have any influence on BMD of women from higher BMI categories. The presence of arterial hypertension or type 2 diabetes increased BMD of lean women, but had no effect on women with higher BMI values [25].

Another study investigated correlations between metacarpal BMD of 532 postmenopausal Japanese women and their body mass index and lifestyle factors. As a result, BMD significantly correlated with BMI and the physical activity index. It did not correlate with calcium and alcohol intake. Also, smoking at the present time did not influence metacarpal BMD value [26].

Chronic alcoholism leads to BMD reduction. Data about caffeine consumption influence on bone metabolism, however, are varied. Caffeine leads to the increase of urinary calcium excretion and possibly reduces calcium absorption in the gastrointestinal tract. According to a considerable number of studies, cigarette smoking leads to the reduction of BMD. In another study, the relation between alcohol, caffeine, past smoking and BMD in a group of 136 Caucasian women was investigated. As a result, an alcohol intake of about 0.5-1 drinks a day correlated positively with BMD. Caffeine was negatively associated with bone density, but it was attenuated with a higher calcium intake. The study did not reveal a significant correlation between past smoking and BMD in multiple regression analyses [27].

Cola intake is associated with a significant decrease of hip but not spine BMD in women. Most kinds of cola contain caffeine, which has negative effects on bone metabolism. Besides, these drinks contain phosphoric acid, which negatively influences calcium absorption and leads to an increase in calcium loss [28].

Fracture risk is determined by both peak bone mass, achieved by the end of adolescence, and bone loss during the aging process. The risk of fracture may be reduced by maximizing peak bone mass. Factors which are positively correlated with the BMD of 8-year-olds include: intake of protein, phosphorus, energy, magnesium, zinc, iron, vitamin K, height and weight [29].

Data revealed that obesity is correlated with increased BMD. The nature of this relationship has not been fully investigated. It may depend on the influence of sex hormones, leptin and insulin. Leptin is produced and secreted mainly through white adipose tissue and correlates with fat mass and body weight. Data suggest that it may also influence osteoblast differentiation and bone matrix mineralization. The study of 100 postmenopausal Turkish women revealed that serum leptin levels did not correlate with BMD of either spine or femoral neck [30]. Another study of 139 postmenopausal women showed significant and positive correlation between serum leptin levels and the BMD of spine and femoral neck [31]. A study of 676 Chinese women aged 20-80 revealed a positive association between serum leptin levels and BMD in the whole group [32].

Data suggest that the serum level of lipids may also be correlated with BMD. The study of pre- and post- menopausal South Korean women showed an inverse association between serum total cholesterol and low density lipoprotein cholesterol level and BMD in the whole group. The level of serum triglycerides had a positive correlation with BMD in the trochanter of femur in post-menopausal women. The concentration of high-density lipoprotein cholesterol in serum had no influence on BMD [33].

Nutrition has an important influence on bone metabolism; however, vitamin D and calcium intake seems to be the most extensive investigated. A study of Italian peri- and postmenopausal women treated with 500 mg calcium and 200 IU vitamin D revealed a significant preventive effect of the supplementation on total BMD in all the women [34]. Another study of 136 Caucasian postmenopausal women showed an independent influence of calcium, energy and protein intake on several bones examined both separately and in multiple regression analysis. Vitamin C, magnesium and zinc intake were correlated with BMD in multiple regression analysis [35]. Another study of men and women aged 65 or older who received supplementation of 500 mg of calcium and 700 IU of cholecalciferol or placebo, showed preventive effects on total, spine and femoral neck BMD in the actively treated group after a period of 3 years [36].

Vitamin A in large amounts is known to have a negative effect on bone growth in mice and humans during foetal bone development. *In vitro* vitamin A increases bone resorption and reduces bone formation. Patients treated with retinoids for skin diseases have modeling abnormalities of long bones calcification of ligaments and osteoporosis. A prospective analysis of postmenopausal women aged 34-77 years showed that women in the highest quintile of total vitamin A intake had significantly elevated relative hip fracture risk compared to those from the lowest quintile [37]. In another cross-sectional study of 175 women aged 28-74, retinol intake was negatively associated with BMD [38].

Few data show an association between vitamin K intake and bone metabolism. Vitamin K may protect against age-related bone loss through vitamin K-dependent carboxylation of bone proteins such as osteocalcin. A cross-sectional study of men and women aged 29-86 revealed that women in the lowest quartile of vitamin K intake had significantly lower spine and femoral neck BMD than those from the highest quartile. On the other hand, no significant association has been found in men [39].

*In vitro* studies suggest that vitamin C is required for type I collagen matrix production, mineralization and expression of osteoblastic markers. The Postmenopausal Estrogen-Progestin Intervenions Trial showed a significant, positive correlation between vitamin C intake and hip BMD. The same correlation, but not significant, was found for spine BMD [40].

Studies suggest that also some of amino acids and their derivatives may affect bone metabolism. Glutamine and its derivative, α-ketoglutarate, play an important role in protein metabolism, amino acids transport across membranes, and the regulation of gene and cellular redox [41]. Dietary α-ketoglutarate is partially converted to energy in enterocytes. It has been proved that up to 20% of it can be recovered in the

blood and is available to take part in metabolic processes in different tissues. Data suggest that dietary α-ketoglutarate acts as a precursor of proline synthesis in intestine, and actively participates in the conversion of proline to hydroxyproline, the main amino acid in collagen [42]. Studies proved that α-ketoglutarate supplementation of pregnant sows, treated with dexamethasone reduced the increase of alkaline phosphatase serum level [43]. Data also report that six-month α-ketoglutarate treatment decreases serum levels of C-terminal cross-linking telopeptide of type I collagen in postmenopausal women with osteopenia [44].

## CONCLUSIONS

Osteoporosis is a common disease which leads to serious complications, such as vertebrae and femur neck fractures. Some factors affecting bone formation and resorption are of genetic origin, others, however, can be modified throughout the lifetime. There are many studies which prove a protective influence of different natural substances on bone metabolism. Data suggest that this can be an effective method of increasing bone formation and inhibiting bone resorption. Prevention of osteoporosis is even more important because of additional effects of antiresorptive drugs and long period of convalescence after bone fracture.

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