

REVIEW

Trace Elements Have Beneficial, as Well as Detrimental Effects on Bone Homeostasis

I. ZOFKOVA¹, M. DAVIS², J. BLAHOS³¹Institute of Endocrinology, Prague, Czech Republic, ²Ecologia Environment, Perth, Australia,³Osteocentrum, Central Military Hospital, Prague, Czech Republic

Received July 31, 2016

Accepted October 27, 2016

On-line February 28, 2017

Summary

The protective role of nutrition factors such as calcium, vitamin D and vitamin K for the integrity of the skeleton is well understood. In addition, integrity of the skeleton is positively influenced by certain trace elements (e.g. zinc, copper, manganese, magnesium, iron, selenium, boron and fluoride) and negatively by others (lead, cadmium, cobalt). Deficiency or excess of these elements influence bone mass and bone quality in adulthood as well as in childhood and adolescence. However, some protective elements may become toxic under certain conditions, depending on dosage (serum concentration), duration of treatment and interactions among individual elements. We review the beneficial and toxic effects of key elements on bone homeostasis.

Key words

Bone mineral density • Bone quality • Boron • Cadmium • Copper • Iron • Fluoride • Lead • Magnesium • Manganese • Selenium • Zinc

Corresponding author

I. Zofkova, Institute of Endocrinology, Narodni 8, 116 94 Prague 1, Czech Republic. E-mail: izofkova@upcmil.cz

Introduction

Bone metabolism is defined by the interaction between osteoclasts, which determine bone resorption, and osteoblasts, which ensure bone formation. Predominance of osteoclastic activity evoked by endocrine, systemic diseases, as well as nutrition state, accelerates bone loss and increases fracture risk. Further

key cell activating bone formation is osteocyte, working first of all *via* mechanoreceptors.

The importance of trace elements in bone regulation is well established. Whereas copper, boron, zinc, selenium, manganese, or magnesium have osteo-protective effects, cadmium, cobalt and lead are toxic. However, the effect of high concentrations of certain bone protective elements (e.g. fluoride) is questionable. A deficit of protective elements, most frequently due to low intake in food, as well as high exposure to toxic elements (e.g. in highly industrial areas) can lead to serious diseases including osteoporosis. This review summarizes contemporary knowledge concerning the positive and/or detrimental effects of trace elements on bone homeostasis. Trace elements are listed in alphabetical order in respective chapter.

Trace elements with prevailing positive effect on the skeleton

Boron

Boron stabilizes cell membranes and modulates membrane transport mechanisms. It has anti-inflammatory, antineoplastic and hypolipidemic effects. It also stimulates bone growth and bone metabolism (Hunt 2012). Boron activates 1,25(OH)₂D₃ production and thus increases bone mineralization (Hakki *et al.* 2013). Adequate boron intake is beneficial for trabecular bone microarchitecture and cortical bone strength (Nielsen and Stoecker 2009). Boron is ubiquitously present in water, soil and plants (the latter

including vegetables, fruits and nuts). It is well tolerated when administered orally and measurable in all tissues. Daily boron intake in adults is usually around 1-2 mg/day. The recommended daily dose to support good bone health is 3 mg (reviewed by Zofkova *et al.* 2013).

Copper

Copper is a general catalytic cofactor, which, both in oxidized and reduced form, affects the redox state in the body. Its deficiency can result in impaired glucose and cholesterol metabolism, energy production, blood and immune cells and altered myocardial contractility (Hordyjewska *et al.* 2014). Copper deficiency leads to idiopathic myelopathy in adults (Kumar *et al.* 2004, Page *et al.* 2015) or progressive peripheral neuropathy (Coyle *et al.* 2015). On the other hand, increased copper levels have been detected in relation to some diseases, such as Menke's disease and Wilson's disease and have some role in formation of plaques in Alzheimer's disease (Brewer 2003).

Copper plays an important role in regulation of bone growth and development of the skeleton. The element induces the formation of lysine crosslinks in collagen and elastin *via* lysyl oxidase activation. As a cofactor of antioxidant enzymes, it removes bone free radicals that cause the osteoclast activation (Kubiak *et al.* 2010). In addition, copper inhibits osteoclastic bone resorption directly (Li and Yu 2007). Altogether, copper increases bone strength and helps to maintain the optimal state of bone quality.

In premature infants between 5 and 6 months of age, copper deficiency was linked to radiographic findings of metabolic bone disease, including osteoporosis, metaphyseal changes, and physal disruptions (Marquardt *et al.* 2012). In consensus with the later observation, is a study performed on osteoporotic post-menopausal women, who had significantly lower serum copper as compared to controls (Midhavi-Roshan *et al.* 2015). Importance of copper in bone regulation also supports the observation of low content of the element in enamel in adult patients with markedly reduced lumbar spine BMD accompanied by severe tooth wear. This phenomenon has been independent of serum 25(OH)D vitamin, PTH, or osteocalcin levels (Sierpinska *et al.* 2014).

In the elderly, physiological decline in the gastrointestinal absorption may induce copper deficiency. Thus, balanced copper homeostasis is undoubtedly of

primary importance for skeletal growth during childhood, as well as for bone health in adult age.

The recommended daily intake of copper for adequate bone quality in adults is 0.9 mg/day (Price *et al.* 2012). Additional clinical studies in larger groups are needed to find conclusive data.

Fluoride

The role of fluoride in the maintenance of bone health is full of controversy. Its positive effect is documented by some *in vitro* studies. Osteoblastic MG-63 cells culture, exposed to fluoride for one week, increased migration of these cells, promoted osteogenic cell differentiation and stimulated ALP concentration in the medium (Ohno *et al.* 2013). In rats, fluoride administration increased expression of mRNA of COL1A1, ALP and Runx2, which could be blocked by DKK-2, an inhibitor of the Wnt/ β -catenin receptor. Thus fluoride stimulates osteoblastogenesis by the canonical Wnt pathway (Pan *et al.* 2014). *In vivo*, the direct effect of fluoride on bone formation is intensified by overproduction of somatomedin. Nevertheless, fluoride treatment did not influence bone strength, although it increased bone mass (Turner *et al.* 1997). The clinical significance of fluoride's bone anabolic effect *in vivo* was questioned, when no association was found between daily intake of fluoride and BMD at lumbar spine and at the hip in a group of adolescents (Levy *et al.* 2014). Similarly, low-dose fluoride had no effect on bone mass and bone metabolic turnover in a relatively large group of post-menopausal women with osteopenia (Grey *et al.* 2013). At excessive exposure levels, intake of fluoride causes skeletal (and dental) fluorosis together with manifestations of gastrointestinal and neurological complications (Jha *et al.* 2011). Some authors explain the detrimental effect of fluoride on the skeleton by an overproduction of parathormone and activated bone resorption (Puranic *et al.* 2015, Koroglu *et al.* 2011).

The sources of fluoride in the environment are fluorite, fluoroapatite and cryolite, as well as compounds of anthropogenic origin, such as coal burning or brick-making industries. Fluoride in food is soluble, 90 % of it is absorbed in the gastrointestinal tract and sequestered mainly in bone and teeth (Jha *et al.* 2011). The variability of fluoride intake in heterogeneous populations is high, ranging from low to toxic values (Chachra *et al.* 2010). Exposure to fluoride could be quantified using urinary and serum fluoride, ALP, bALP and BGP, as demonstrated in animals (Song *et al.* 2011).

Iron

As an enzymatic cofactor, iron stimulates synthesis of bone matrix *via* activation of lysyl hydroxylase. Iron also activates 25-hydroxycholecalciferol hydroxylase and supports mineralization of bone matrix through vitamin D. Rats suffering from a severe iron deficiency had poorly mineralized skeleton (Parelsman *et al.* 2006) together with lower cortical width in femur and tibia, pathological changes in the microarchitecture of the vertebral trabecular bone and decreased bone strength (Madeiros *et al.* 2004). In addition, severe iron deficiency in rats was associated with a decline in biochemical markers of bone formation, such as procollagen type I N-terminal propeptide, which was normalized after a diet with adequate iron content (Diaz-Castro *et al.* 2012). Thus, iron appears to be a key nutrient necessary for integrity of the skeleton of adult animals. Besides, low iron status activates gene expression of FGF23, the molecule with a more common pathogenetic role in living organisms.

The bone-protective effect of iron has also been shown in a clinical study of patients with iron-deficiency anemia. Pre-menopausal women with non-recovered anemia had, after adjusting for age and BMI, significantly higher levels of bone resorption marker (NTx) and slightly lower bone formation marker (P1NP) compared to treated women (Wright *et al.* 2013). Therefore, bone resorption dominates over bone formation in pre-menopausal women with iron deficiency. However, osteopenia was also observed in patients with extremely high iron concentrations in tissues, e.g. due to genetically determined hemochromatosis (Guggenbuhl *et al.* 2005). In excess, ferric ion activates osteoclastic differentiation, most probably through activation of marrow-derived macrophages. Oxidative stress (e.g. induced by hypoestrogenism) may play a supporting role in this process (Xiao *et al.* 2015). In summary, the protective or destructive effects of iron on bone are a matter of tissue concentration. This phenomenon was also observed in some other trace elements. An adequate intake of iron has not been established with respect to bone mineral density.

Magnesium

About 60 % of total magnesium is stored in the skeleton. Magnesium is an integral part of the apatite crystals, from which it is released in the course of bone resorption. It is a cofactor of a number of enzymes that are important for energy (ATP synthesis), lipid, protein and nucleic acid, as well as calcium metabolism

(reviewed by Castiglioni *et al.* 2013). Magnesium deficiency is associated with gastrointestinal or renal diseases, sickle cell anemia and diabetes and it is also known to impede long-term antineoplastic and diuretic treatment. Low magnesium level alters cardiac excitability and neuromuscular function. Magnesium deficiency is often found in alcoholics and patients with Alzheimer disease, and elderly people with hypertension and cerebrovascular accidents (Volpe 2013). Hypomagnesemia is often found in patients after kidney transplantation due to increased renal magnesium wasting. It can increase mortality in patients with cardiovascular disease and/or cause graft dysfunction and disturbance of immunity (van Lacede and van Biesen 2015).

In the skeleton, magnesium supports production of hydroxyapatite (Aina *et al.* 2013) and bone marrow stromal cells mineralization (Yoshizawa *et al.* 2014). Magnesium also supports 1,25(OH)₂D vitamin synthesis. Thus, magnesium deficiency *via* hypocalcaemia elevates parathormone synthesis and subsequently osteoclast activity. A stimulated release of inflammatory cytokines, such as tumor necrosis factor alpha (TNF α) – and interleukin-1 (Rude *et al.* 2009), may also play a potential role in this process. On the other hand, osteoblastic number and activity declines during magnesium deficiency (Kanazawa *et al.* 2007). Altogether, the consequence of magnesium deficiency is accelerated bone loss, which, together with decline in bone formation, leads to decreased trabecular volume and alteration of bone microarchitecture in a way similar to osteomalacia.

Clinical studies indicate that sufficient magnesium intake increases bone density in young adults (Matias *et al.* 2012). The protective effect of high magnesium intake on bone quality was documented in healthy women, using ultrasound measurement of the calcaneus (BUA) (Kim *et al.* 2011). An extensive analysis by Hayhoe *et al.* (2015) in a large group of adult subjects of both genders showed that dietary magnesium and serum magnesium were positively associated with calcaneal bone ultrasound analysis in women and negatively associated with fracture risk in both women and men. Furthermore, magnesium supported facilitated postoperative healing in orthopedic patients, in whom degradable fixation magnesium devices were used (Chaya *et al.* 2015). Thus, magnesium appears to be an important element for development of peak bone mass, as well as for integrity of the adult skeleton (Nieves 2013).

Nevertheless, some alarming data exist on negative effects of elevated serum magnesium on the skeleton. Hypermagnesemia (e.g. in chronic renal failure) disturbs calcium/magnesium ratio, which may lead to a defect in mineralization and osteoblast differentiation (Leidi *et al.* 2011). Warning results were also revealed by the Women's Health Initiative Study, where in a group of more than 89,000 postmenopausal women, subjects with the highest quintile of magnesium intake also had the highest incidence of wrist fractures. This phenomenon may be, in fact, caused by deterioration of bone quality in subjects exposed to high magnesium concentration.

Apart from nutrition, genetics is also likely to be responsible for magnesium balance. This hypothesis stems from results of some association studies. Runnels (2011) found association between magnesium status and polymorphism TRPM6 in humans. A meta-analysis of genome-wide association studies (GWAS) showed an association between serum magnesium levels and variants at six genomic loci: MUC1, ATP2B1, DCDC5, TRPM6, SHROOM3 and MDS1. In addition, some of these genes were also associated with bone mineral density (Meyer *et al.* 2010). These observations may provide some new insights into the role of endogenous magnesium in the regulation of bone mass.

Subclinical magnesium deficiency has been found in most populations of Europe and North America due to relatively low intake of micronutrients concentrated in green vegetables, nuts or grains. Besides, magnesium absorption also varies with other dietary elements, such as phosphate, which forms insoluble complexes with magnesium. The recommended daily intake of magnesium necessary for bone health in young people under 30 years of age is 400 mg, in older subjects it is 320 mg and 420 mg for females and males, respectively. In practice, magnesium supplementation is required in all subjects with low magnesium levels (Nieves 2013).

Manganese

Manganese is an essential trace element, which plays a role in lipid and carbohydrate metabolism. In the skeleton, manganese positively modulates RANKL/OPG ratio in the process of bone formation, determining thickness of trabecular bone area and increasing trabecular number (Liu *et al.* 2015). Local delivery of manganese chloride (MnCl₂) increased significantly the maximum torsion rigidity and blood vessel density in the subperiosteal region in rats at day 10 post fracture (Hrdna

et al. 2015). From this perspective, local MnCl₂ is a pharmaceutical with a potential to support fracture healing.

Like zinc, manganese accelerates growth (at least partly) *via* activation of somatomedin synthesis (reviewed by Zofkova *et al.* 2013). In oophorectomized rats, those supplemented with manganese had slower bone loss than rats without supplementation (Rico *et al.* 2000). The amount of manganese in bones of rats decreased after oophorectomy and normalized during estradiol treatment (Rahnama *et al.* 2003). The direct bone-protective effect of estrogen on bone metabolism is, therefore, intensified by manganese deposition.

Using an absorption spectrophotometric method, we found a positive association between serum manganese levels and bone mineral density, and a negative correlation between serum manganese and the number of fractures in a group of 40 post-menopausal women (Nemcikova *et al.* 2009). These results might suggest that manganese has some protective effect on bone health in hypoestrogenic women. These results were obtained in a small sample size, however, and should be confirmed by further investigations.

Nevertheless, manganese can also be toxic to humans. With its half-life of about 8-9 years, manganese accumulates substantially in bones. Long-term overexposure to manganese, with subsequent increase in manganese blood levels, may lead to dopaminergic dysfunction, which manifests as Parkinson's disease (O'Neal and Zheng 2015, Sánchez-González *et al.* 2015).

Finally, in physiological concentrations, manganese has a significant bone protective effect. The monitoring of manganese homeostasis is recommended in patients with a high risk of osteoporosis, although the toxic effect of supraphysiological concentrations should not be underestimated. The recommended daily intake of manganese is 1.8 – 2.3 mg (Devrian and Volpe 2003).

Selenium

Selenium is an essential nutrient necessary for human and animal health due to a strong antioxidant action, which protects cells (including those in the skeleton) from oxidative damage. It is known that selenium deficiency activates bone resorption. Detrimental effects on bone microarchitecture were documented in mice (Cao *et al.* 2012) and on bone mineral density and growth in rats (Moreno-Reyes *et al.* 2001). In healthy humans, selenium status was negatively correlated with bone metabolic turnover and positively

with bone density (Hoeg *et al.* 2012, reviewed by Zeng *et al.* 2013). A large study in a group of 2,374 euthyroid post-menopausal women showed that selenium levels positively correlated with hip and/or lumbar spine BMD and negatively with bone remodelling (Hoeg *et al.* 2012). Moreover, disabled elderly patients had low selenium and zinc levels, together with impaired total antioxidant score and low BMD (Younesi *et al.* 2015).

As mentioned above, skeletal effect of selenium is explainable by its antioxidative mechanism. In low antioxidative status, osteoclasts produce high amount of ROS, which is accompanied by activated bone resorption. Selenium substitution restores antioxidant capacity in bone cells and inhibits NF- κ B – RANKL axis and osteoclast differentiation. In high doses, selenium induces apoptosis of mature osteoclasts. In addition, selenium strengthens osteoblast antioxidative defense. Through these mechanisms, selenium modulates bone metabolic turnover in favor of bone formation (reviewed by Zeng *et al.* 2013).

In summary, selenium appears to be a potent antioxidant element with a protective effect on the skeleton by maintaining cell redox balance. Nevertheless, a study by Arikan *et al.* (2011) failed to confirm any correlation between serum selenium and bone mass. A further, large investigation is, therefore, necessary to confirm possible use of selenoproteins in treatment of osteoporosis. The recommended daily dose of selenium is 55 μ g/day. An alarming fact is that in Europe, Africa and Asia the usual selenium intake is only around 25 μ g/day (Price *et al.* 2012).

Zinc

Zinc is known to regulate growth, neuronal development and immunity (Plum *et al.* 2010). Zinc positively influences the strength, flexibility and architecture of the skeleton in animals. The bone anabolic effect of the element was documented by an increase in osteocalcin and COL1A1 expression and serum alkaline phosphatase activity. Recently, zinc has also been shown to have beneficial effect on biomechanical bone parameters in rats (Bortolin *et al.* 2015). Osteoporotic post-menopausal women had significantly lower serum zinc levels than healthy controls (Midhavi-Roshan *et al.* 2015). Similarly, disabled elderly patients had significantly lower circulating zinc together with an impaired antioxidant score and lower bone mineral density, as compared to age-gender-matched controls (Younesi *et al.* 2015).

The bone protective effect of zinc is complex. The element is a growth-stimulator through activation of enzymes, which, support synthesis of DNA, RNA and proteins. Zinc increases osteoblastic activity and promotes synthesis of collagen. On the other hand, zinc inhibits osteoclastic bone resorption and thus disconnects bone remodelling in favor of bone formation (Lowe *et al.* 2002, reviewed by Zofkova *et al.* 2013). A study in adolescent rats suggested that severe zinc deficiency could have negative implications for future bone health (Ryz *et al.* 2009).

In addition, in zinc deficient rats serum PTH levels increased as a consequence of inhibition of calcium absorption in the intestine (Suzuki *et al.* 2015). Furthermore, zinc stabilizes the membrane of mast cells. Atik *et al.* (2006), on the basis of *in vitro* studies and observation in patients with senile osteoporosis, outlined the hypothesis on the role of hyperparathyroidism evoked by zinc deficiency, degranulation of mast cells, heparin release and increase in activity of prostaglandin E₂. Heparin, together with prostaglandin E₂, are the cofactors of parathormone, which intensifies the direct effect of zinc deficiency on bone resorption.

The favorable effect of zinc intake on bone remodelling, bone matrix formation and mineralization was demonstrated in growing rats (Headley *et al.* 2010). A study in humans showed positive correlation between serum zinc and osteocalcin and negative correlation between zinc and bone resorption markers (Hill *et al.* 2005). Similarly, zinc supplementation increased bone formation in premenarcheal girls (Berger *et al.* 2015).

Zinc has a beneficial effect on bone integrity throughout life. Newborn animals that were exposed to zinc deficiency had reduced production of somatomedin (IGF-I), the parameter important for initiating a growth spurt. However, decline in somatomedin synthesis also deteriorates bones in elderly people and contributes to the development of osteopenia and alteration of bone microarchitecture (reviewed by Zofkova *et al.* 2013). Therefore, zinc supplementation appears to have an important role in treatment and prevention of senile osteoporosis.

Zinc also protects the skeleton of alcoholics, which comes mainly from activation of anabolic processes in bone (Yamauchi *et al.* 2006). The administration of zinc to rats that were exposed to alcohol increased bone formation and the volume of trabecular bone (Gonzalez-Reimers *et al.* 2005). Zinc also reduces negative effects of toxic elements, such as lead or

cadmium on osteoblasts (Jamieson *et al.* 2006, Brzóska *et al.* 2007).

The human body contains 2 – 3 g of zinc, and its average daily loss (approx. 0.1 %) would be normally covered by dietary intake. However, this is not always the case, and approximately 25 % of the world's population is at risk of zinc deficiency, especially adolescents and postmenopausal women (Meunier *et al.* 2005). Zinc deficiency was associated with greater impairment in bone development than protein-caloric restriction, and it limited bone recovery during repletion in growing rats (Hosea *et al.* 2004). While the recommended daily minimum intake of zinc is 12 mg, 15 mg is necessary to increase bone density (Maret and Sanstead 2006). However, it should be noted that long-term administration of very high doses of zinc, particularly if the patient also has an existing copper deficiency, can lead to chronic zinc toxicity (Palacios 2006). Considering the above, zinc in physiological concentrations has an important bone-protective effect. Its deficiency leads not only to deterioration of growth, but also alters integrity of adult skeletons in humans and animals.

Trace elements extremely detrimental to the skeleton

Cadmium

Cadmium is soft, extremely toxic element found in high concentrations in industrial areas. It is mostly a by-product of mining and metal processing. The progress of industrialization leads to an increased risk of cadmium pollution as cadmium cannot be destroyed in nature. In high concentrations, cadmium induces oxidative stress, which causes aberrant DNA methylation, alters cell proliferation and differentiation, inhibits DNA damage repair and destabilizes the genome (Bishak *et al.* 2015). In other words, cadmium is a strong carcinogenic element. Moreover, when administered for a long time *via* inhalation, the element leads to pulmonary fibrosis and renal disorder.

Cadmium also seriously damages bone mass and bone quality. Its toxic effect on the skeleton has been demonstrated in a correlation study in a group of 850 postmenopausal women, in whom the bone density value negatively correlated with cadmium concentration in urine (Akeson *et al.* 2006). Engström *et al.* (2012) studied a group of nearly 3,000 postmenopausal women and found a negative association between cadmium intake and BMD at the total body and the lumbar spine,

and a positive relationship with fracture risk, even when the cadmium exposure was relatively low. This is in consensus with the result of a small study, in which women with severe osteoporosis showed cadmium concentration in plasma significantly higher than control women (Sadeghi *et al.* 2014). Positive association between urinary calcium and cadmium content in kidney specimens obtained from biopsy and negative correlation between kidney cadmium and bone mineral density in women were observed by Wallin *et al.* (2013).

Cadmium has a negative impact on growth. Exposure to the element during pregnancy negatively influences birth size in neonates. In Bangladeshi women, maternal urinary cadmium inversely correlated with neonatal birth weight and head and chest circumferences in girls (Kippler *et al.* 2012). In 155 schoolchildren in Pakistan, two times higher cadmium concentration in urine was associated with 1.72 times increase in deoxy pyridin and a 1.21 times increase in calcium excretion. This association, however, needs to be analyzed in other ethnic groups, too.

The mechanism of detrimental cadmium effect on the skeleton is not fully understood. *In vitro* study showed that longer exposure of human osteoblast-like cells to cadmium induced cytoskeleton disruption and accelerated cell apoptosis (Papa *et al.* 2015). Liang *et al.* (2015) found an elevated urinary β_2 -microglobulin, which is a serious indicator of tubular dysfunction, in women subjected to long-term exposure to cadmium in the most polluted areas. Furthermore, Kim *et al.* (2014) found low values of BMD in exposed females, together with high urinary β_2 -microglobulin. The tubular dysfunction (probably as a consequence of activated fibroblast growth factor 23) appears to be the fundamental cause of calcium-phosphate imbalance in cadmium-exposed subjects (Kido *et al.* 2014).

However, while an inverse correlation between urinary cadmium and BMD was found in men exposed to high cadmium concentrations, only 5 % of them showed evidence of renal tubular disorder with urine β_2 -microglobulin exceeding the value of 300 $\mu\text{g/g}$ creatinine (Nawrot *et al.* 2010). Thus, the latter study shows that cadmium's negative influence on the skeleton is independent of the tubular effect. *In vivo*, cadmium inhibits bone formation and stimulates bone resorption, even at moderate concentrations. In toxic concentrations, cadmium breaks down the collagen matrix and inhibits its mineralization (reviewed by Zofkova *et al.* 2013). The detrimental effect of cadmium on metabolism of

beneficial trace elements such as zinc, iron, magnesium, selenium, copper, and manganese should be also taken into account.

Heavy metals (lead and chromium)

Lead accumulates in bones over a long time and damages osteoblastogenesis. Beier *et al.* (2015) demonstrated a detrimental effect of long-term exposure to lead on bone accrual and bone strength in juvenile male mice. Women with higher lead content in their skeleton had thinner cortices in distal tibias and lower volumetric BMD (Wong *et al.* 2015). Furthermore, the latter parameter in the same gender negatively correlated with urinary lead levels (Tsai *et al.* 2015).

Chromium has a potentially harmful effect on bone health *via* oxidative stress. The element reduced osteoblast-like cell survival and activity *in vitro* in several different concentrations and incubation times, and decreased OPG/RANKL ratio (mainly due to stimulation of osteoclasts forming) (Zijlstra *et al.* 2012, Andrews *et al.* 2011). An imbalance in bone remodelling that favors resorption may contribute to complications following total hip arthroplasty. Relatively limited data exist on the detrimental effect of lead and chromium on the skeleton, therefore further investigations are necessary.

Conclusions

Many trace elements (e.g. boron, iron, magnesium, and manganese) are beneficial for bone health; however some are toxic, such as cadmium, cobalt and lead. It is important to note, nevertheless, that excessive intake of certain beneficial elements (fluoride, iron, magnesium, zinc) can also have toxic effects. For example, high intake of fluoride damages bone integrity. The positive or negative effect of trace elements depends,

to a degree, on the influence of external environments (nutrition) and internal factors (individual absorption and metabolism of these elements, genetic disposition, age and gender). Finally, the effect of trace elements depends not only on their quantity in the diet but also on their mutual interactions (Khandare *et al.* 2005).

Perspectives in the topic research

The protective effect of selected trace elements on the skeleton has been confirmed; however theoretical and practical issues of supplementation in terms of osteoporosis prevention and treatment are still unknown. Data on the effect of individual elements are limited, partly because these elements are usually taken in combinations. In addition, homeostasis of trace elements in the body is determined by diet, as well as age and gender (Sakai *et al.* 2000). Due to these inconsistencies, further studies are necessary to confirm the effect of different doses, concentrations and interactions of individual trace elements on bone health. From this perspective, absorption spectrophotometry remains a valuable tool in measuring trace elements concentrations in body fluids (blood and urine) and tissues and, therefore, it contributes to the prevention and treatment of osteoporosis.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the project (Ministry of Health, Czech Republic) for conceptual development research organization 00023761 (Institute of Endocrinology, Prague, Czech Republic).

References

- AINA V, LUSVARDI G, ANNAZ B, GIBBON IR, IMRIE FE, MALAVASI G, MENABUE L, CARRATO G, MARTRA G: Magnesium - and strontium-co-substituted hydroxyapatite: the effects of doped-ions on the structure and chemico-physical properties. *J Mater Sci Mater Med* **23**: 2867-2979, 2012.
- AKESSON A, BJELLERUP P, LUNDH T, LIDFELDT J, NERBRAND C, SAMSIOE G, SAMSIOE G, SKERFVING S, VAHTER M: Cadmium-induced effects on bone in a population-based study of women. *Environ Health Perspect* **114**: 830-834, 2006.
- ANDREWS RE, SHAH KM, WILKINSON JM, GARTLAND A: Effects of cobalt and chromium ions at clinically equivalent concentrations after metal-on-metal hip replacement on human osteoblasts and osteoclasts: implications for skeletal health. *Bone* **49**: 717-723, 2011.

- ARIKAN DC, COSKUN A, OZER A, KILINC M, ATALAY F, ARIKAN T: Plasma selenium, zinc, copper and lipid levels in postmenopausal Turkish women and their relation with osteoporosis. *Biol Trace Elem Res* **144**: 407-417, 2011.
- ATIK OS, USLU MM, EKSIÖGLU F, SATANA T: Etiology of senile osteoporosis. a hypothesis. *Clin Orthopaed Related Res* **443**: 25-27, 2006.
- BERGER PK, POLLOCK NK, LAING EM, CHERTIN V, BERNARD PJ, GRIDER A, SHAPSES SA, DING KH, ISALES CM, LEWIS RD: Zinc supplementation increases procollagen type 1 amino-terminal propeptide in premenarcheal girls: a randomized controlled trial. *J Nutr* **145**: 2699-2704, 2015.
- BIER EE, INZANA JA, SUDU TJ, SHU L, PUZAS JE, MONEY RA: Effects of combined exposition to lead and high-fat diet on bone quality in juvenile mice. *Environ Health Perspect* **123**: 935-943, 2015.
- BISHAK YK, PAYAHOO L, OSATDRAHIMI A, NOURAZARIAN A: Mechanisms of cadmium carcinogenicity in the gastrointestinal tract. *Asian Pac J Cancer Prev* **16**: 9-21, 2015.
- BORTOLIN RH, DA GRACA AVEZEDO AREU BJ, ABBOLTT CALVAO URURAHY M, COSTA DE SOUZA KS, BEZERRA JF, LOUREIRO MB, DA SILVA FS, MARQUES DE, BATISTA AA, OLIVEIRA G, ET AL.: Protection against T1DM-induced bone loss by zinc supplementation: biomechanical, histomorphometric, and molecular analyses in STZ-induced diabetic rats. *PLoS One* **10**: e0125349, 2015.
- BREWER GJ: Copper in medicine. *Curr Opin Chem Biol* **7**: 207-212, 2003.
- BRZÓSKA MM, ROGALSKA J, GALAZYN-SIDORCZUK M, JURCZUK M, ROSZCZENKO A, KULIKOWSKA-KARPIŃSKA E, MONIOSZKO-JAKONIUK J: Effect of zinc supplementation on bone metabolism in male rats chronically exposed to cadmium. *Toxicology* **237**: 89-103, 2007.
- CAO JJ, GREGOIRE BR, ZENG H: Selenium deficiency decreases antioxidative capacity and is detrimental to bone microarchitecture in mice. *J Nutr* **142**: 1526-1531, 2012.
- CASTIGLIONI S, CAZZANIGA A, ALBISETTI W, MAIER JA: Magnesium and osteoporosis: current state of knowledge and future research directions. *Nutrients* **5**: 3022-3033, 2013.
- CHACHRA D, LIMEBACK H, WILLETT TL, GRYNPAS MD: The long-term effects of water fluoridation on the human skeleton. *J Dent Res* **89**: 1219-1223, 2010.
- CHAYA A, YOSHIZAWA S, VERDELIS K, MYERS N, COSTELLO BJ, CHOU DT, PAL S, MAITI S, KUMTA PN, SFEIR C: In vivo study of magnesium plate and screw degeneration and bone fracture healing. *Acta Biomater* **18**: 262-269, 2015.
- COYLE L, ENTEZARALMAHDI M, ADEOLA M, DE HOYOS P, MEHED A, VARON J: The perfect storm: copper deficiency presenting as progressive peripheral neuropathy. *Am J Emerg Med*. **34**: 340.e5-340.e6, 2016.
- DEVIRIAN TA, VOLPE SL: The physiological effects of dietary boron. *Crit Rev Food Sci Nutr* **43**: 219-231, 2003.
- DIAZ-CASTRO J, LÓPEZ-FRIAS MR, CAMPOS MS, LÓPEZ-FRIAS M, ALFÉREZ MJ, NESTARES T, OJEDA ML, LÓPEZ-ALIAGA I: Severe nutritional iron-deficiency anaemia has a negative effect on some bone turnover biomarkers in rats. *Eur J Nutr* **51**: 241-247, 2012.
- DIAZ-CASTRO J, RAMÍREZ LÓPEZ-FRIAS M, CAMPOS MS, LÓPEZ-FRIAS M, ALFÉREZ MJ, NESTARES T, ORTEGA E, LÓPEZ-ALIAGA I: Goat milk during iron repletion improves bone turnover impaired by severe iron deficiency. *J Dairy Sci* **94**: 2752-2761, 2011.
- ENGSTRÖM A, MICHAËLSSON K, VATER M, JULIN B, WOLK A, ÄKESSON A: Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone* **50**: 1372-1378, 2012.
- FAYHOE RP, LENTJES MA, LUBEN RN, KHAW KT, WELCH A: Dietary magnesium and potassium intakes and circulating magnesium are associated with heel bone ultrasound attenuation and osteoporotic fracture risk in the EPIC-Norfolk cohort study. *Am J Clin Nutr* **102**: 376-384, 2015.
- GONZALEZ-REIMERS E, DURAN-CASTELLON M, MARTIN-OLIVERA R, POLEZ-RAMÍREZ A, GARCIA-VALDECASAS COPMPELO E: Effects of zinc supplementation on ethanol-mediated bone alterations. *Food Chem Toxicol* **43**: 1497-1505, 2005.
- GREY A, GARG S, DRAY M, PURVIS L, HORNE A, CALLON K, GAMBLE G, BOLLAND M, REID IR, CUNDY T: Low-dose fluoride in postmenopausal women: a randomized controlled trial. *J Clin Endocrinol Metab* **98**: 2301-2307, 2013.

- GUGGENBUHL P, DEUGNIER Y, BOISDET JF, ROLLAND Y, PERDRIGER A, PAWLOTSKY Y, CHALES G: Bone mineral density in men with genetic hemochromatosis and HFE gene mutation. *Osteoporosis Int* **16**: 1809-1814, 2005.
- HAKKI SS, DUNDAR N, KAYIS SA, HAKKI EE, HAMURCU, KERIMOGLU U, BASPINAR N, BASOVOU A, NIELSEN FH: Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. *J Trace Elem Med Biol* **27**: 148-153, 2013.
- HEADLEY KB, NEWMAN SM, HUNT JR: Dietary zinc reduces osteoclast resorption activities and increases markers of osteoblast differentiation, matrix maturation, and mineralization in the long bones of growing rats. *J Nutr Biochem* **21**: 297-303, 2010.
- HILL T, MEUNIER N, ANDRIOLLO-SANCHEZ M, CIARAPICA D, HININGER-FAVIER I, POLITO A, O'CONNOR JM, COUDRAY C, CASHMAN KF: The relationship between the zinc nutritive status and biochemical markers of bone turnover in older European adults: the ZENITH study. *Eur J Clin Nutr* **59** (Suppl 2): S73-S78, 2005.
- HOEG A, GOGAKOS A, MURENY E, MUELLER S, KÖHRLE J, REID DM, GLÜER CC, FELSEBERG D, ROUX C, EASTELL R, SCHONBURG L, WILLIAMS GR: Bone turnover and bone mineral density are independently related to selenium status in healthy euthyroid postmenopausal women. *Clin Endocrinol Metab* **97**: 4061-4070, 2012.
- HORDYJEWSKA A, POPIOLEK L, KOCOT J: The many „faces“ of copper in medicine and treatment. *Biometals* **27**: 611-621, 2014.
- HOSEA HJ, TAYLOR CG, WOOD T, MOLLARD R, WEILER HA: Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. *Exp Biol Med (Maywood)* **229**: 303-311, 2004.
- HREHA J, WEY A, CUNNINGHAM C, KRELL ES, BRIETBART EA, PAGLIA DN, MONTEMURRO NJ, NGUYEN DA, LEE YJ, KOMLOS D, ET AL.: Local manganese chloride treatment accelerates fracture healing in a rat model. *J Orthop Res* **33**: 122-130, 2015.
- HUNT CD: Dietary boron: progress in establishing essential roles in human physiology. *J Trace Elem Med Biol* **26**: 2-3, 2012.
- JAMIESON JA, TAYLOR CG, WEILER HA: Marginal zinc deficiency exacerbates bone lead accumulation and high dietary zinc attenuates lead accumulation at the expense of bone density in growing rats. *Toxicol Sci* **2006**: 286-249, 2006.
- JHA SK, MISHRA VK, SHARMA DK, DAMODARAN T: Fluoride in the environment and its humans. *Rev Environ Contam Toxicol* **211**: 121-142, 2011.
- KHANDARE AL, SURESH P, KUMAR PU, LAKSMAIAH N, MANULA N, RAO G: Beneficial effect of copper supplementation on deposition of fluoride in bone in fluoride - and molybdenum-fed rabbits. *Calcif Tissue Int* **77**: 233-238, 2005.
- KIDO S, FUJIHARA M, NOMUR K, SASAKI S, MUKAI R, OHNISHI R, KANEKO I, SEGAWA H, TATSUMI S, IZUMI H, KOHNO K, MIYAMOTO K: Molecular mechanisms of cadmium-induced fibroblast growth factor 23 upregulation in osteoblast-like cells. *Toxicol Sci* **139**: 301-316, 2014.
- KIM YD, YIM DH, EOM SY, MOON SI, PARK CH, KIM GB, YU SD, CHOI BS, PARK JD, KIM H: Differences in the susceptibility to cadmium-induced renal tubular damage and osteoporosis according to sex. *Environ Toxicol Pharmacol* **38**: 272-278, 2014.
- KIPPLER M, TOFAIL F, GARDNER R, RAHMAN A, HAMADANI JD, BOTTAI M, VAHTER M: Maternal cadmium exposure during pregnancy and size at birth: a prospective cohort study. *Environ Health Perspect* **120**: 284-289, 2012.
- KOROGLU BK, ERSOY IH, KOROGLU M, BAKALI A, ERSOY S, VAROL S, TAMER MN: Serum parathyroid hormone levels in chronic endemic fluorosis. *Biol Trace Elem Res* **143**: 79-86, 2011.
- KUBIAK K, KLIMCZAK A, DZIKI L, MODRANKA R, MALINOWSKA K: Influence of copper (II) complex on the activity of selected oxidative enzymes. (In Polish) *Pol Merkur Lekarski* **28**: 22-25, 2010.
- KUMAR N, GROSS JB JR, AHLKOG JE: Copper deficiency myelopathy produces a clinical picture like subacute combined degeneration. *Neurology* **63**: 33-39, 2004.

- LI BB, YU SF: In vitro study of the effects of copper ion on osteoclastic resorption in various dental mineralized tissues. *Zhoghua Kou Qiang Yi Xue Za Zhi* **42**: 110-113, 2007.
- LEIDI M, DELLERA F, MARIOTTI M, MAIER JA: High magnesium inhibits human osteoblast differentiation in vitro. *Magnes Res* **24**: 1-6, 2011.
- LEVY SM, WARREN JJ, PHIPPS K, LETUCHY E, BROFFITT B, EICHENBERGER-GILMORE J, BURNS TL, KAVAND G, JANZ KF, TORNER JC, PAULEY CA: Effects of life-long fluoride intake on bone measures of adolescents: a prospective cohort study. *J Dent Res* **93**: 353-359, 2014.
- LIANG Y, LEI L, NILSSON J, LI H, NORDBERG M, BERNARD A, NORDBERG GF, BERGDAHL IA, JIN T: Renal function after reduction in cadmium exposure: an 8-year follow – up of residents in cadmium-polluted areas. *Environ Health Perspect* **120**: 223-228, 2015.
- LIU R, JIN C, WANG Z, WANG Z, WANG J, WANG L: Effects of manganese deficiency on the microstructure of proximal tibia and OPG/RANKL gene expression in chicks. *Vet Res Commun* **39**: 31-37, 2015.
- LOWE NM, FRASER WD, JACKSON MJ: Micronutrient group symposium on micronutrient supplementation: is there a case? Is there a potential therapeutic value of copper and zinc for osteoporosis? *Proc Nutr Soc* **61**: 181-185, 2005.
- MADEIROS DM, STOECKER B, PLATTNER A, JENNINGS D, HAUB M: Iron deficiency negatively affects vertebrae and femurs of rats independently of energy intake and body weight. *J Nutr* **34**: 3061-3067, 2004.
- MARET W, SANDSTEAD HH: Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol* **20**: 3-18, 2006.
- MARQUARDT ML, DONE SL, SANDROCK M, BERDON WE, FELDMAN KW: Copper deficiency presenting as metabolic bone disease in extremely low birth weight, short-gut infants. *Pediatrics* **130**: e695-e698, 2012.
- MEUNIER N, O'CONNOR JM, MAIANI G, CASHMAN KD, SECKER DL, FERRY M, ROUSSEL AM, COUDRAY C: Importance of zinc in the elderly: the ZENITH study. *Eur J Clin Nutr* **59** (Suppl 2): S1-S4, 2005.
- MEYER TE, VERWOERT GC, HWANG SJ, GLAZER NL, SMITH AV, VAN ROOIJ FJ, EHRET GB, BOERWINKLE E, FELIX JF, LEAK TS, ET AL.: Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six loci influencing serum magnesium levels. *PLoS Genet* **6**: e1001045, 2010.
- MIDHAVI-ROSHAN M, EBRAHIMI M, EBRAHIMI A: Cooper, magnesium, zinc and calcium status in osteopenic and osteoporotic post-menopausal women. *Clin Cases Miner Bone Metab* **12**: 18-21, 2015.
- MORENO-REYES R, EGRISE D, NÈVE J, PASTEELS JL, SCHOUTENS A: Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. *J Bone Miner Res* **16**: 1556-1563, 2001.
- NAWROT T, GEUSENS P, NULENS TS, NEMERY B: Occupational cadmium exposure and calcium excretion, bone density, and osteoporosis in men. *J Bone Miner Res* **25**: 1441-1445, 2010.
- NEMCIKOVA P, SPEVACKOVA V, CEJCHANOVA M, HILL M, ZOFKOVA I: Relationship of serum manganese and copper levels to bone density and quality in postmenopausal women. A pilot study. *Osteol Bull* **14**: 97-100, 2009.
- NIELSEN FH, STOECKER B: Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone. *J Trace Elem Med Biol* **23**: 195-203, 2009.
- NIEVES JW: Skeletal effects of nutrients and nutraceuticals, beyond calcium and vitamin D. *Osteoporos Int* **24**: 771-786, 2009.
- OHNO M, KIMOTO K, TOYODA T, KAWATA K, ARAKAWA H: Fluoride-treated bio-resorbable synthetic nonceramic (corrected) hydroxyapatite promotes proliferation and differentiation of human osteoblastic MG-63 cells. *J Oral Implantol* **39**: 154-160, 2013.
- O'NEAL SL, ZHENG W: Manganese toxicity upon overexposure: a decade in review. *Curr Environ Health Rep* **2**: 315-328, 2015.
- PAGE PS, NAZAR RG, PARK MC, JAMES RF: Copper deficiency myelopathy in the setting of advanced degenerative cervical spondylosis. *Br J Neurosurg*. **30**: 453-455, 2016.
- PALACIOS C: The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutr* **46**: 621-628, 2006.

- PAN L, SHI X, LIU S, GUO X, ZHAO M, CAI R, SUN G: Fluoride promotes osteoblastic differentiation through canonical Wnt/ β -catenin signaling pathway. *Toxicol Lett* **225**: 34-42, 2014.
- PAPA V, BIMONTE VM, WANNENES F, D'ABUSCO AS, FITTIPALDI S, SCANDURRA R, POLITI L, CRESCIOLI C, LENZI A, DI LUIGI L, MIGLIACCIO S: The endocrine disruptor cadmium alter human osteoblast-like Saos-2 cells homeostasis in vitro by alteration of Wnt/ β -catenin pathway and activation of caspases. *J Endocrinol Invest* **38**: 1345-1356, 2015.
- PARELMAN M, STOECKER B, BAKER A, MEDEIROS D: Iron restriction negatively affects bone in female rats and mineralization of hFOB osteoblast cells. *Exp Biol Med (Maywood)* **231**: 378-386, 2006.
- PLUM LM, RINK L, HAASE H: The essential toxin: impact of zinc on human health. *Int J Environ Res Public Health* **7**: 1342-1365, 2010.
- PRICE CT, LANGFORDT JR, LIPORACE FA: Essential nutrients for bone health and a review of their availability in the average north american diet. *Open Orthop J* **6**: 143-149, 2012.
- PURANIK CP, RYAN KA, YIN Z, MARTINEZ-MIER EA, PREISSER JS, EVERETT ET: Fluoride modulates parathyroid hormone secretion in vivo and in vitro. *Cells Tissues Organs* **200**: 413-423, 2015.
- RAHNAMA M, BLONIARZ J, ZAREBA S, SWIATKOWSKI W: Study of estrogen deficiency impact on manganese levels in teeth and mandible of rats after ovariectomy. *Rocz Panstw Zakl Hig* **54**: 33-38, 2003.
- RICO H, GÓMEZ-RASO N, SEVILLA M, HERNÁNDFEZ ER, SECO C, PÁEZ E, CRESPO E: Effects on bone loss of manganese alone or with copper supplement in ovariectomized rats. a morphometric and densitometric study. *Eur J Obstet Gynecol Reprod Biol* **90**: 97-101, 2000.
- RUDE RK, SINGER FR, GRUBER HE: Skeletal and hormonal effects of magnesium deficiency. *J Am Coll Nutr* **28**: 131-141, 2009.
- RUNNELS LW: TRPM6 and TRPM7: a Mul-TRP-PLIK-cation of channel functions. *Curr Pharm Biotechnol* **12**: 42-53, 2011.
- RYZ NR, WEILER HA, TAILOR CG: Zinc deficiency reduces bone mineral density in the spine of young adult rats: a pilot study. *Ann Nutr Metab* **54**: 218-26, 2009.
- SADEGHI N, OVEISI MR, NAHNAT B, HAJIMAHMOODI M, BEHZAD M, BEHFAR A, SADEGHI F, SAADATMAND S: The relationship between bone health and plasma zinc, copper, lead and cadmium concentration in osteoporotic women. *J Environ Health Sci Eng* **12**: 125, 2014.
- SAKAI T, WARIISHI M, NISHIYAMA K: Changes in trace element concentrations in hair of growing children. *Biol Trace Elem Res* **77**: 43-51, 2000.
- SÁNCHEZ-GONZÁLEZ C, LÓPEZ-CHAVES C, GÓMEZ-ARACENA J, GALINDO P, ARANDA P, LLOPIS J: Association of plasma manganese levels with chronic renal failure. *J Trace Elem Med Biol* **31**: 78-84, 2015.
- SIERPINSKA T, KONSTANTYNOWICZ J, ORYWAL K, GOLEBIEWSKA M, SZMITKOWSKI M: Copper deficit as a potential pathogenic factor of reduced bone mineral density and severe tooth wear. *Osteoporos Int* **25**: 447-454, 2014.
- SONG YE, TAN H, LIU KJ, ZHANG YZ, LIU Y, LU DL, TU J, CUI CY: Effect of fluoride exposure on bone metabolism indicators ALP, BALP, and BGP. *Environ Health Prev Med* **16**: 158-163, 2011.
- SUZUKI T, KAJITA Y, KATSUMATA S, MATSUZAKI H, SUZUKI K: Zinc deficiency increases serum concentration of parathyroid hormone through a decrease in serum calcium and induces bone fragility in rats. *J Nutr Sci Vitaminol (Tokyo)* **5**: 382-390, 2015.
- TSAI TL, PAN WH, CHUNG YT, WU TN, TSENG YC, LIOU SH, WANG SL: Association between urinary lead and bone health in a general population from Taiwan. *J Expo Sci Environ Epidemiol* **26**: 481-487, 2016.
- TURNER CH, GARETTO LP, DUNIPACE AJ, ZHANG W, WILSON ME, GRYNPAS MD, CHACHRA D, McCLINTOCK R, PEACOCK M, STOOKEY GK: Fluoride treatment increased serum IGF-1, bone turnover, and bone mass, but not bone strength, in rabbits. *Calcif Tissue Int* **61**: 77-83, 1997.
- VAN LAECKE S, VAN BIESEN W: Hypomagnesaemia in kidney transplantation. *Transplant Rev (Orlando)* **29**: 154-160, 2015.
- VOLPE SL: Magnesium in disease prevention and overall health. *Adv Nutr* **4**: 378S-383S, 2013.

-
- WALLIN M, SALLSTEN G, FABRICIUS-LAGGING, E, OHRN C, LUNDH T, BARREGARD L: Kidney cadmium levels and association with urinary and bone mineral density: a cross-sectional study in Sweden. *Environ Health* **12**: 22, 2013.
- WONG AK, BEATTIE KA, BHARGAVA A, CHEUNG M, WEBBER CE, CHETTLE DR, PAPAIOANNOU A, ADACHI JD, CANADIAN MULTICENTRE OSTEOPOROSIS STUDY (CaMOS) RESEARCH GROUP: Bone lead (Pb) content at the tibia is associated with thinner distal tibia cortices and lower volumetric bone density in postmenopausal women. *Bone* **79**: 58-64, 2015.
- WRIGHT I, BLANCO-ROJO R, FERNÁNDEZ MC, TOXQUI L, MORENO G, PÉREZ-GRANADOS AM, DE LA PIEDRA C, REMACHA ÁF, VAQUERO MP: Bone remodelling is reduced by recovery from iron deficiency anaemia in premenopausal women. *J Physiol Biochem* **69**: 889-896, 2013.
- XIAO W, BIEBEI D, GUANGSI X, YU J, WEN Z, XI H, YOUJIA X: Iron overload increased osteoclastogenesis and aggravates the effects of ovariectomy on bone mass. *J Endocrinol* **226**: 121-134, 2015.
- YAMAGUCHI M, UCHIYAMA S, HASHIMOTO K: Oral administration in combination with zinc enhances β -cryptoxanthin-induced anabolic effects on bone components in the femoral tissues of rats in vivo. *Biol Pharm Bull* **29**: 371-374, 2006.
- YOSHIZAWA S, BROWN A, BRCHOWSKY A, SFEIR C: Magnesium ion stimulation of bone marrow stromal cells enhances osteogenic activity, stimulating the effect of magnesium alloy degradation. *Acta Biomater* **10**: 2834-2342, 2014.
- YOUNESI S, PARSIAN H, HOSSEINI SR, NOREDDINI H, MOSAPOUR A, BIJANI A, HALALKHOR S: Dyshomeostasis of serum oxidant/antioxidant status and copper, zinc, and selenium levels in elderly physically disabled persons: an AHAP-based study. *Biol Trace Elem Res* **166**: 136-141, 2015.
- ZENG H, CAO JJ, COMBS GF JR: Selenium in bone health: roles in antioxidant protection and cell proliferation. *Nutrients* **5**: 97-110, 2013.
- ZIJLSTRA WP, BULSTRA SK, VAN RAAIJ JJ, VAN LEEUVEN BM, KUIJER R: Cobalt and chromium ions reduce human osteoblast-like cell activity in vitro, reduce the OPG to RANKL ratio, and induce oxidative stress. *J Orthop Res* **30**: 740-747, 2012.
- ZOFKOVA I, NEMCIKOVA P, MATUCHA P: Trace elements and bone health. *Clin Chem Lab Med* **51**: 1555-1561, 2013.
-