Vitamin K and bone metabolism

Vitamina K şi metabolismul osos

Daniela Fodor[1*], Adriana Albu[1], Laura Poantă[1], Laura Munteanu[2]

1. 2nd Internal Medicine Clinic, 2. Rheumatology Clinic,
"Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Vitamin K has a well known role in the synthesis of a number of blood coagulation factors. During the last decade, new reports about the importance of the vitamin K for bone and vascular health were published. Osteocalcin, the most important noncollagenous protein of the bone, is a vitamin K-dependent protein and provides a measure of the vitamin K status of the bone. Recent data demonstrated a correlation between vitamin K intakes, bone mass, osteoporosis and risk of fracture. Human intervention studies have shown that vitamin K supplementation increases the bone mineral density in osteoporotic patients and reduces fracture rates. Also there are evidences about a synergistic effect of vitamin K and vitamin D in bone metabolism. The aim of this paper is to present the impact of vitamin K upon bone health.

Key words: vitamin K, osteocalcin, bone health
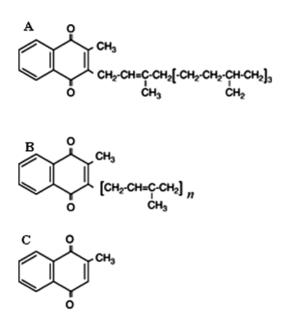
Rezumat

Rolului tradiţional al vitaminei K în sinteza unora dintre factorii coagulării i s-au adăugat în ultimii ani o serie de dovezi cu privire la implicarea acesteia în sănătatea osoasă şi vasculară. Osteocalcina, proteina necolagenă majoritară din ţesutul osos, dependentă de vitamina K este considerată a reprezenta un marker valoros al statusului vitaminei K. Studii recente au demonstrat existenţa unei relaţii între aportul de vitamină K, masa osoasă, osteoporoză şi riscul de fractură, suplimentarea cu vitamina K ducând la creşterea densităţii minerale osoase la pacienţii osteoporotici şi scăderea ratei fracturilor. De asemenea exista dovezi despre acţiunea sinergică a vitaminei K şi a vitaminei D asupra metabolismului osos. Aceată lucrarea îşi propune o trecere în revistă a datelor recente din literatură cu privire la implicarea vitaminei K în sănătatea osoasă.
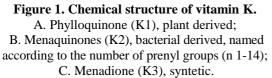
Cuvinte cheie: vitamina K, osteocalcina, sănătatea osoasa

[*]**Corresponding author:** Fodor Daniela Clinica Medicală II, 2-4 Clinicilor Str 400006, Cluj-Napoca, Romania, Tel: 0745381603, E-mail dfodor@umfcluj.ro

Introduction

Vitamin K was discovered in 1920 by the Danish biochemist Henrik Dam. He received the Nobel Prize in Medicine in 1943 for his work on vitamin K. The term "K" is derived from the German „*koagulation-vitamin*" referring to its role in the process of blood clot formation. Recently, advances in the discovery and characterization of new vitamin K-dependent proteins have led to the conclusion that vitamin K plays different roles in bone and vascular health. There are two natural forms of vitamin K, vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones), and a synthetic compound-vitamin K_3 or menadione also exists *Figure 1)*. The major source of vitamin K_1 is leaf vegetables (collards, spinach, salad greens, broccoli, and cabbage) and some vegetable oil and margarines (oil from soybean, canola, cottonseed or olive). Menaquinones (of which a potential source are bacteria from the large intestine) are classified depending of the length of their side-chain (abbreviated to MK-*n*): the most nutritionally relevant are MK-4 (from meats, milk products, eggs) and MK7-9 (from animal livers, cheeses and natto) (1-3). As shown in *Table 1* there are different dietary sources of vitamin K_1 and vitamin K_2 (4). The adequate intake level for vitamin K is considered to be 90 µg for women and 120 µg for men (1).

In all cells that synthesize vitamin K-dependent proteins, vitamin K quinine, derived from dietary sources, is transformed in vitamin K quinol (KH_2). KH_2 acts as a cofactor for the endoplasmic reticulum enzyme γ-glutamil carboxylase, for posttranslational carboxylation of glutamate residues into γ-carboxy glutamate (Gla) (*Figure 2*). These Gla-residues are essential for stabilizing the tertiary structure and for the function of the containing proteins (Gla-proteins) (3,5). Gla-proteins' family includes several blood coagulation factors synthesized in the liver (II, VII, IX, X), osteocalcin (OC) synthesized in bone and matrix Gla-protein (MPG) synthesized in extra hepatic tissues, especially in cartilage and arterial wall (6,7). The characterization of these proteins has demonstrated the implication of vitamin K not only in blood coagulation but also in bone metabolism, atherosclerosis and more recently in inflammation, oxidative stress, nerve signaling and even renal calculi (2,8).

Due to the influence of vitamin K in bone metabolism and the rising importance of the osteoporosis, this paper is a review of the recent evidences about the connection between vitamin K and bone health.

Physiology of bone metabolism and vitamin K involvement

The normal bone, a tissue with a continuous turnover, results from the balance between the activity of bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts) that have a permanent bone-modeling activity. In normal conditions the forming and resorbing

Figure 1. Chemical structure of vitamin K.
A. Phylloquinone (K1), plant derived;
B. Menaquinones (K2), bacterial derived, named according to the number of prenyl groups (n 1-14);
C. Menadione (K3), syntetic.

Tabel 1. Vitamin K1 and K 2 content of food sources (according to Schurgers et al [4])

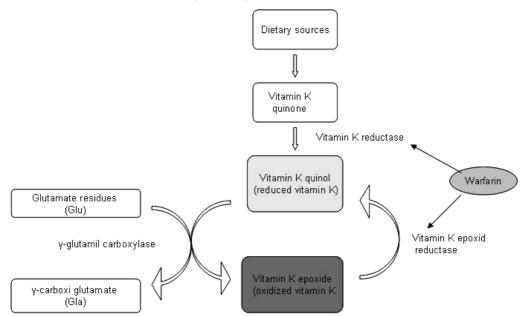| Food source	Vitamin K1 (µg/100g)	Vitamin K2 (µg/100g)
Meat	0.5-5	1-30
Fish	0.1-1	0.2-4
Fruit	0.1-3	-
Green vegetables	100-700	-
Grains	0.5-3	-
Natto	20-40	900-1200
Cheese	0.5-10	40-90
Eggs	0.5-2.5	10-25
Margarine and plant oils	50-200	-

activities are coupling and thus the bone formation is equal with bone resorption. Unbalances of bone turnover appear in the periods of growing or senescence.

Bone remodeling can be assessed by measuring surrogate markers of bone turnover in the blood or urine (*Table 2*).

The level of these markers may identify changes in bone remodeling within a relatively short time interval (several days to months) before changes in bone mineral density can be detected (9,10).

Vitamin K is a potential contributory factor in the regulation of bone remodeling. This regulation is intermediated mainly by OC, but there are recent evidences for association

Figure 2. The vitamin K cycle. Vitamin K quinone, from dietary sources, is reduced to vitamin K quinol by vitamin K reductase. Vitamin K quinol is then oxidized to vitamin K epoxide in a reaction coupled to γ-carboxylation of glutamic acid residues (Glu) to γ-carboxi glutamate (Gla). Vitamin K epoxide is then reduced to vitamin K quinol in a reaction catalyzed by vitamin K epoxid reductase. Warfarin or other coumarin-related compounds act as an inhibitor of the reductases and disrupt the recycling of vitamin K.

Table 2. Biomarkers of bone turn-over.

Markers of bone formation
    Bone alkaline phosphatase (bone ALP)
    Osteocalcin (OC)
    N-terminal propeptides of type-1 procollagen (P1NP)
    C-terminal propeptides of type-1 procollagen (P1CP)

Markers of bone resorption
    Pyridinoline (PYD)
    Deoxypyridinoline (DPD)
    C-terminal telopeptide of type-1 collagen (CTX)
    N-terminal telopeptide of type-1 collagen (NTX)

between protein S and Gas-6 (another Gla-proteins) and low bone mass (3). In addition to its function as an enzymatic cofactor, vitamin K acts as an anabolic agent stimulating the synthesis of osteoblasts and as an inhibitor of osteoclasts formation, decreasing their bone resorptive activity (11). Also, vitamin K induces osteoclast apoptosis, inhibits osteoblast apoptosis and shifts the balance toward bone deposition (12,13).

OC is an abundant non-collagenous bone matrix protein, (the second most abundant protein in the bone matrix, over 20%) and is synthesized by mature osteoblasts and in a smaller quantity by osteocytes and odontoblasts. OC messenger RNA has also been detected in tissues other than bone, but it appears to be processed properly only in the bone microenvironment. The structure of OC is characterized by the presence of three glutamic acid residues, which undergo a vitamin K-dependent carboxylation. The γ-carboxyglutamic acid residues (Gla) provide osteocalcin with the ability to bind with high affinity calcium from hydroxyapatite (8) the result of its action being the attraction of calcium ions (Ca^{2+}) and the incorporation these ions into the hydroxyapatite crystals (14). The production of OC is regulated by vitamin K and vitamin D. Vitamin D promotes the transcription of the OC gene, whereas vitamin K promotes the posttranscriptional carboxylation of Gla residues in the osteocalcin propeptide (15). It was demonstrated that 1,25-dihydroxyvitamin D_3, the most active metabolite of vitamin D_3, enhances the activity of γ-glutamyl carboxylase (suggesting that the carboxylation of OC is stimulated by vitamin D) (16) and that menaquinone-4 stimulates 1,25-dihydroxyvitamin D_3-induced mineralization by human osteoblasts (17).

Determination of phylloquinone plasma levels is possible, but its utility in assessing vitamin K status is insufficient, due to the influence of non-dietary factors such as plasma triglycerides and smoking status that needs to be accounted for (18). Assays for serum and urine vitamin K do not provide information on adequacy at the tissue level (3). Prothrombin time is only useful in screening bleeding abnormalities due to deficiency of vitamin K dependent coagulation factors. In exchange, the circulating concentration of under- γ-carboxylated OC (ucOC) is consider to be a sensitive marker of vitamin K status, especially when expressed as a ratio to carboxylated OC (ucOC/cOC) (19). Population studies suggest a correlation between levels of circulating vitamin K, ucOC, and cOC; all three parameters should be measured for determining the real status of vitamin K (3). The plasma level of phylloquinone is an indicator of recent dietary phylloquinone intake but the serum percentage of ucOC is considered to be a sensitive marker of vitamin K availability in bone (18).

The exact role of OC in bone metabolism is less known. Due to its high affinity for hydroxyapatite it seems to be required to stimulate bone mineral maturation (10). On the other hand, an increase in bone formation without impairing bone resorption was demonstrated in osteocalcin-deficient mice, indicating OC as a negative regulator of bone formation (20). The high bone mass loss in OC deficient mice after ovariectomy, suggests the protective effect of OC in post-menopausal period(21). The study of Ivaska et al (22) demonstrated that osteocalcin is released during bone resorption as well as produced by osteoblasts during bone formation, and the bone matrix-derived OC may contribute to circulating OC

levels. The authors propose that serum osteocalcin should be considered as a marker of bone turnover rather than bone formation.

Protein S also has functions unrelated to anticoagulation. Only the free form of circulated Protein S (40% of total protein S) acts as a cofactor for anticoagulation [23]. The remainder of 60% circulates bound to C4BP, a regulator of the complement system. This complex acts as limiter for complement activation involved in the clearance of apoptotic cells by phagocytosis (24). The phagocytosis of apoptotic cells seems to limit the inflammation and to prevent autoimmune disorders. It was demonstrated that Protein S is a neuroprotective factor during ischemic brain injury and is an important autocrine substance for vascular smooth muscle cells (25,26). Protein S deficiency has been associated with osteopenia and osteonecrosis, suggesting an additional role in bone metabolism (27). Protein S shares an extensive amino acid sequence with another vitamin K-dependent protein, Gas6 (for Growth arrest-specific gene 6) (26). Gas6 is expressed ubiquitously in heart, lung, stomach, kidney, muscle, brain, spleen, liver, ovary, and testis. In these locations it seems to increase the proliferation and the survival of fibroblasts and vascular smooth muscle cells (29). In interaction with Axl (the receptor tyrosine kinase) Gas 6 may modulate cell survival, differentiation, adhesion, migration, and/or proliferation of articular cartilage, rheumatoid arthritic synovium/ synovial fluid, and the neointima of arteries after balloon injury (30).

Like the others Gla-protein family members, MGP contains Gla residues which justify its high affinity for calcium; the attachment of these residues is done via gama-carboxylation, a process that is inhibited by warfarin (7,31,32). The importance of MGP for bone and cartilage metabolism is underlined by the phenotype of MGP-deficient mice. These mice have shown tachycardia, short stature and prematurely died at 2 months. The direct cause of death in these mice was the rupture of thoracic and abdominal aorta, with severe hemorrhage. The alizarin red staining proved an extensive vascular calcification in the early postnatal stage (2-3 weeks of life) and the aorta wall of these mice displayed foci of cartilaginous metaplasia (33). The skeletal phenotype of the MPG-deficient mice is characterized by calcification of the cartilage in the proliferating chondrocytes zone, lack of organization in columns of these cells, and absence of hypertrophic chondrocytes. These findings indicate an important deficiency of enchondral ossification, and explain the short stature and osteopenia (33). The mechanisms proposed for explaining the inhibition of calcification process by MPG are related to the binding of calcium in the nucleating hydroxyapatite (inhibiting the crystal growth), and to the blocking of the action of a bone morphogenetic protein (BMP-2), a potent factor of bone maturation (34,35). MGP modulates mesenchymal cell differentiation, and the absence of BPM inhibition leads to the differentiation of vascular mesenchyme cells into bone cells, thus increasing calcification (35).

The intervention of vitamin K in bone metabolism is also related to its effect on the calcium balance, as calcium is a key mineral in the bone metabolism. A diet rich in vitamin K (natto eaters) was associated with a lower urinary calcium excretion than that of non natto eaters (36). Due to the importance of calcium supplementation in attaining a high peak bone mass and reducing postmenopausal bone loss, further data is required to understand the connection between vitamin K and calcium (37).

Vitamin K intake and bone mass

There are several factors that influence the peak bone mass. This notion reflects the skeleton age, and the Dual energy X-ray absorptiometry (DXA) measurements established the peak bone mass between 26-30 years (9). The factors are endogenous (genetic and hormonal) and exogenous (nutrition and lifestyle). Despite the fact that endogenous factors, especially genetic factors, are responsible for 70%

of the bone mass, there are a numerous studies about dietary factors involved in the bone health. Nutrition is a factor that could be influenced in the prophylaxis of the osteoporosis, and excepting calcium and vitamin D, there are other components to consider. Recent studies and accumulating evidences about vitamin K influence on the bone metabolism through OC suggest that physicians should encourage their patients to consume more food rich in vitamin K (2).

In newborn babies, the classic deficiency of vitamin K becomes apparent in the first week of life and includes intra-abdominal and intracranial hemorrhage or cephalic hematoma. The low transplacental transfer, the absence of intestinal bacteria, and the low concentration of vitamin K in human milk are some of the factors responsible for this deficiency. This is the reason why the prophylaxis with vitamin K is indicated in newborn babies, especially in preterm infants (38).

Even if there are obvious evidence that phylloquinone intakes in many children and adolescents are below the recommended level (1, 39-42), there are only few studies about the influence of vitamin K on bone health in children and teenagers. Understanding the role of vitamin K in bone metabolism in children is important because the maximization of the accretion of bone mass during growth could be an important strategy for the minimization of the risk for osteoporosis in the elder years. Better vitamin K status (high plasma phylloquinone and low %ucOC) was associated with decreased bone turnover and with changes of the lumbar spine in healthy young girls (but not in the hip) (43). A recent study (44) in pre-pubertal girls (11-12 years) shows that a better vitamin K status, assessed as a low %ucOC, was associated with increased bone mineral content (total body and lumbar spine), but not bone turnover. Also, the %ucOC was inversely correlated with serum 25-hydroxyvitamin D levels, the most widely used marker of vitamin D status. The presented findings suggest potentially adverse effects of low vitamin K status for bone health in-

dices, but there are no evidences to provide cause and effect. There is a need for more trials to confirm the need for regular supplementation of vitamin K in children and adolescents.

Serum %ucOC as well as serum 25-hydroxyvitamin D concentrations exhibited seasonal variation (with lowest values of the first and the highest values of the second in late summer/early autumn). This variation was demonstrated in the young adolescents (44) and in the elderly women (45) but more research is needed to define the relationship between vitamin K and vitamin D status.

There are several studies in which the investigation of the association between daily vitamin K intake and serum concentration of ucOC demonstrated an inverse correlation (46-49) in adults. High serum levels of ucOC were associated with low bone mineral density (BMD) in postmenopausal women (50,51), and low plasmatic levels of vitamin K with low BMD in the spine (52). The differences between male and female BMD and vitamin K status are related to the estrogen status. Among men, poor vitamin K nutritional status was associated with low BMD at the hip, while in postmenopausal women not using estrogen replacement it was associated with low BMD of the spine. These associations were not observed among the pre- and postmenopausal women using estrogen replacement (18). Also, a low serum ucOC concentration was found to be a marker for hip fracture in the elderly population, although the mechanism by which ucOC is linked to bone fragility is yet unclear (45). A prospective cohort study (EPIDOS) drew the same conclusion; the level of serum ucOC, but not total OC, predicts hip fracture risk independently of femoral neck BMD in elderly women (53).

Vitamin K is not known to have a carrier protein; triglyceride-rich lipoproteins are considered to be the main form to transport vitamin K to osteoblasts. Despite this fact, adjustment for triglycerides is not necessary when examining associations between vitamin K and bone health (18).

The age is inversely correlated with %ucOC and with the ratio between ucOC and intact OC (ucOC/iOC). The plasmatic level of K_1 or MK-7 necessary for minimize the ucOC concentration is higher in the group aged over 70 years, and it decreases progressively for each of the younger age groups (54). Although the precise role of ucOC remains unclear, these age- related differences should have an importance in a possible vitamin K supplementation.

Vitamin K intake supplementation and its influence on bone health

In the last years several prospective studies have evaluated the effect of vitamin K supplementation on the bone metabolism, especially regarding the protection against the bone loss. Vitamin K_1 or vitamin K_2, mainly MK-4 and MK-7, were used in order to ensure the supplementation.

The study of Braam et al (55) investigated the effects of daily supplementation for three years of vitamin D and minerals comparative with vitamin D, minerals and 1000 µg vitamin K_1 on healthy postmenopausal women. The authors had measured BMD and biochemical markers of bone formation and resorption. No significant differences were observed among the groups with respect to BMD change in the lumbar spine. If co-administered with minerals and vitamin D, vitamin K_1 may substantially contribute to reducing postmenopausal bone loss with 30-40% in the femoral neck. If the observed effect sustains during longer periods of intake, it may result in postponement of osteoporosis to later ages.

Bolton-Smith et al. (56) performed a 2-year double-blind, placebo-controlled trial of supplementation in healthy women aged 60 years and older (four groups: placebo, vitamin D+calcium, vitamin D+calcium+200 µg vitamin K_1 and 200 µg vitamin K_1 alone). They found no significant changes from baseline in BMD at the femoral site but the women who took com-

bined vitamin K, vitamin D, and calcium had a significant increase in BMD at the ultradistal radius, a site consisting mainly of trabecular bone (but not at the lumbar spine or total body). The authors suggest the hypothesis of a synergistic effect of this combination. They assume that any synergy between vitamin K and D derives from separate effects exerted independently or alternatively from their concerted action through common proteins (OC, matrix Gla protein, and Gas6) or pathways. The hypothesis of the direct influence of vitamin D on the γ-carboxylation reaction of bone Gla proteins was not supported by their findings. In this study the γ-carboxylation of OC was neither enhanced by vitamin D nor by its combination with vitamin K. Recently, Booth et al. (57) in a 3-year, double-blind, controlled trial on 60-80 years subjects (500 µg vitamin K_1+ multivitamin compared to multivitamin, plus calcium and vitamin D) found no differences in BMD measurements at any of the anatomical sites (hip, lumbar spine, and total body) measured between the two groups. The same non-significant improvement of BMD was found at the hip and lumbar spine in the study of Volpe at al. (58) after supplementation for 6 months with 600 µg vitamin K in pre- and perimenopausal women.

In a recent meta-analysis of randomized controlled trials with vitamin K supplementation for longer than 6 months for reducing bone loss and preventing fractures, Cockayne et al. (59) suggests that supplementation with vitamin K_1, and menaquinone, especially MK-4, reduces the bone loss and fractures, particularly hip fractures among Japanese patients. It has been not established yet whether K_1 or MK-4 has the most anti-osteoporotic effect. The only difference between both vitamins is the aliphatic side chain, which does not influence the coenzyme function, but which does affect the distribution of vitamin K over the lipoproteins in the blood, and the transport to and absorption by the various tissues (60). The combination between vitamin K_2 and risedronat had additive

effects on osteocyte density and lacunar occupancy, and also a synergistic effect on glucocorticoid-induced cortical porosity (61)

In the randomized controlled trial ECKO (62), the supplementation with vitamin K1 was 2-4 years long. No protection against age-related decline in BMD was found, but this dose may reduce the incidence of fractures and cancers in postmenopausal women with osteopenia. The anticancer effects of vitamin K could be mediated through tyrosinkinaze (63).

Vitamin K antagonists and bone health

Oral anticoagulants are used today in the treatment of an increased number of patients with thrombosis or with risk of thrombosis. Their effects are related to the inhibition of vitamin K reductase and vitamin K epoxide reductase in hepatocytes and osteoblasts, resulting in vitamin K deficiency. The impact of vitamin K antagonists on bone health remains controversial (64) but accumulating evidence has shown that warfarin increases fracture risk by impairing bone quality (65). Thus, warfarin appears to induce skeletal fragility through decrease in bone OC content. This hypothesis was evaluated in rats by Sugiyama *et al* (66). They demonstrated that long-term warfarin administration did not change BMD, but markedly decreased osteocalcin content, diminished mineral size, and impaired material hardness in humeral cortical bone.

A recent study (67) in children under long-term oral anticoagulant therapy shows a disturbance of the equilibrium between the bone formation and resorption by favoring the latter. In these cases the authors recommend for the physician to monitor the bone status and to only intervene with appropriate nutritional or pharmacological regimens when necessary.

These data raise the question of the necessity of other type of anticoagulant treatment (prothrombin specific inhibitors or X factor inhibitors) in order to avoid the negative effects of the warfarin especially in patients with osteoporosis.

Conclusions

In the past decade it has become evident that vitamin K plays multiple and significant roles in human health, others that its function in blood coagulation. There are evidences about the implication of vitamin K especially in the bone health and the vascular calcification. These functions are accomplished mainly through Gla-protein family, with OC having an important role in bone metabolism. OC should be considered as a marker of bone turnover.

Accumulated evidence indicates that subclinical non-hemostatic vitamin K deficiency in extrahepatic tissues, particularly in bone and possibly in blood vessels, exists widely in the otherwise healthy adult population. The available data suggest that a daily intake of 200-500 µg of vitamin K may be required for optimal gamma-carboxylation of OC. The same benefits may be achieved by a daily supplementation with 100 µg vitamin K, but more studies are needed to further examine the consequences of continuous administration of vitamin K.

The human intervention studies demonstrated that vitamin K can increase BMD in osteoporotic patients and can reduce fracture rate. There are growing evidences about the synergistic effect on bone health of vitamin K, vitamin D, calcium, and possible others micronutrients. Further data is required in order to have a complete understanding of the complex interaction between vitamin K, vitamin D and bone metabolism.

Acknowledgements

References

1. Food and Nutrition Board Institute of Medicine. Dietary reference intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium,, and Zinc. National Academy Press, Washington, DC, USA, 2001
2. Ryan-Harshman M, Aldoori W. Bone health. New role for vitamin K? Can Fam Physician 2004;50:993-997
3. Vermeer C, Shearer MJ, Zittermann A, et al. Beyond

deficiency: Potential benefits of increased intakes of vitamin K for bone and vascular health. Eur J Nutr 2004;43:325-335

4. Schurgers LJ, Geleijnse JM, Grobbee DE, et al. Nutritional intake of vitamin K1 (phylloquinone) and K2 (menaquinone) in the Netherlands. J Nutr Environ Med 1999;9:115-122

5. Spronk HMH, Soute BAM, Schurgers LJ, et al. Matrix Gla protein accumulated at the border of regions of calcification and normal tissue in the media of the arterial vessel well. Biochem Biophys Res Commun 2001;289:485-490

6. Braam LA, Hoekes Ap, Brouns F, et al- Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. Thromb Haemost 2004;91:373-380

7. Hofbauer LC, Brueck CC, Shanahan CM, et al. Vascular calcification and osteoporosis- from clinical observation towards molecular understanding. Osteoporos Int 2007;18:251-259

8. Shearer MJ, Newman P. Metabolism and cell biology of vitamin K. Thromb Haemost 2008;100:530-547

9. Bolosiu HD. Biologia sistemului osos. In: Bolosiu HD. Osteoporoza. Casa Cărţii de Stiinţă, Cluj-Napoca 2008, 13-20

10. Seibel MJ. Biochemical markers of bone turnover. Part I: biochemistry and variability. Clin Biochem Rev 2005;26:97-122

11. Koshihara Y, Hoshi K, Okawara R, et al. Vitamin K stimulates oseteoblastogenesis and inhibit osteoclastogenesis in human bone marrow cell culture. J Endocrinol 2003;176:339-348

12. Urayama S, Kawakami A, Nakashima T, et al. Effect of the vitamin K2 on osteoblasts apoptosis: vitamin K2 inhibit apoptotic cell death of human osteoblasts induced by Fas, proteasome inhibitor, etoposide, and staurosporine. J Lab Clin Med 2000;136:181-193

13. Kameda T, Miyazama K, Mori Y, et al. Vitamin K2 inhibits osteoclastic bone resorption by inducing osteoclasts apoptosis. Biochem Biophys Res Commun 1996;220:515-519

14. Bügel S. Vitamin K and bone health. Proc Nutr Soc 2003;62:839-843 (abstr)

15. Furie B, Furie BC. Molecular basis of vitamin K-dependent γ-carboxylation. Blood 1990;75:1753-1762

16. Miyake N, Hoshi K, Sano Y, et al. 1,25-Dihydroxyvitamin D3 promotes vitamin K2 metabolism in human osteoblasts. Osteoporos Int 2001;12:680-687

17. Koshihara Y, Hoshi K, Ishibashi H, et al. Vitamin K2 promotes 1α,25(OH)2 vitamin D3-induced mineralization in human periosteal osteoblasts in vitro. J Bone Miner Res 1997;12:431-438

18. Booth SL, Broe KE, Peterson JW, et al. Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab 2004;89:4904-4909

19. McCormick RK Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility. Altern Med Rev. 2007;12:113-45.

20. Ducy P, Desbois C, Boyce B. Increased bone formation in osteocalcin-deficient mice. Nature. 1996;382:448-52

21. Blanqué R, Cottereaux C, Gardner CR. Increases in osteocalcin after ovariectomy are amplified by LPS injection: strain differences in bone remodeling. Gen Pharmacol 1998;30:51-6

22. Ivaska KK, Hentunen TA, Vääräniemi J, et al. Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. J Biol Chem 2004;279:18361-18369

23. Berkner KL, Runge KW. The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. J Tromb Haemost 2004;2:2118-2132

24. Webb JH, Blom AM, Dahlbäck B. Vitamin K-dependent protein S localizing complement regulator C4b-binding protein to the surface of apoptotic cells. J Immunol 2002;169-2580-2586

25. Liu D, Guo H, Griffin JH, et al. Proteins S confers neuronal protection during ischemic/hypoxic injury in mice. Circulation 2003;107:1791-1796

26. Benzakour O, Formstone C, Rahman S, eta l- The anticoagulant factor, protein S produced by cultured human vascular smooth muscle cells and its expression is up-regulated by thrombin. Blood 2000;95:2008-2014

27. Pierre-Jacques H, Glueck CJ, Mont MA, et al. Familial heterozygous protein-S deficiency in a patient who had multifocal osteonecrosis. A case report. J Bone Joint Surg 1997;79:1079-1084

28. Manfioletti G, Brancolini C, Avanzi G, Schnieder C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related of protein S, a negative coregulator in the blood coagulation cascade. Mol Cell Biol 1993;13:4976-4985

29. Collett G, Wood A, Alexander MY, et al. Receptor tyrosin kinase Axl modulates the osteogenic differentiation of pericytes. Circ Res 2003;92:1193-1199

30. Katagiri M, Hakeda Y, Chikazu D, et al- Mecanisms of stimulation of osteoclastic bone resorption through Gas6/Tyro 3, a receptor tyrosin kinase signaling, in mouse osteoclasts. J Biol Chem 2001;276:7376-7382

31. Anagnostis P, Karagiannis A, Kakafika AI, et al. Atherosclerosis and osteoporosis: age dependent degenerative processes or related entities? Osteopor Int 2009;20:197-207

32. Shanahan CM. Mechanisms of vascular calcification in renal diseases. Clin Nephrol 2005;63:146-157

33. Luo G, Ducy P, McKee MD, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997;386:78-81

34. Danilevicius CF, Lopes JB, Pereira RMR. Bone metabolism and vascular calcification. Braz J Med Biol Res, 2007;40:435-442

35. Boström K, Tsao D, Shen S, et al. Matrix Gla protein modulates differentiation induced by bone morphogeneic protein-2 in C3H10T1/2 cells. J Biol Chem 2001;276:14044-14052

36. Sakamoto N, Nishiike T, Iguchi H, Sakamoto K. The effect of diet on blood vitamin K status and urinary mineral excretion assessed by a food questionnaire. Nutr Health 1999;13:1-10

37. Lanham-New SA. Importance of calcium, vitamin D and vitamin K for osteoporosis prevention and treatment. Proc Nutr Soc 2008 ;67:163-176

38. Van Winckel M, De Bruyne R, Van De Velde S, Van Biervlit S. Vitamin K, an update for the paediatrician. Eur J Pediatr 2009;168:127-134

39. Booth SL, Pennington JA, Sadowski JA. Food sources and dietary intakes of vitamin-K-1 (phylloquinone) in the American diet:data from PDA Total Diet Study. J Am Diet Assoc 1996 ;96:149-154

40. Collins A, Cashman KD, Kiely M. Vitamin K1 intake and status in Irish adolescent girls. Int J Vitamin Nutr Res 2006;76:385-390

41. Pyrnne CJ, Thane CW, Prentice A, Wadsworth MEJ. Intake and sources of phylloquinone (vitamin K1) in 4-year-old British children: comparation between 1950 and the 1990s. Publ Health Nutr 2005;8:171-180

42. Thane CW, Pyrnne CJ, Ginty F, et al. Dietary phylloquinone (vitamin K1) intake: comparison between adolescents living in Cambridge and a national British sample. Proc Nutr Soc 2002;61:146A

43. Kalkwarf HJ, Khbury JC, Bean J, Elliot JG. Vitamin K, bone turnover, and bone mass in girls. Am J Clin Nutr 2004;80:1075-1080

44. O'Connor E, Mølgaard C, Michaelsen KF, et al. Serum percentage undercarboxylated osteocalcin, a sensitive measure of vitamin K status and its relationship to the bone health indices in Danish girls. Br J Nutr 2007;97:661-666

45. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxyilated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 1993;91:1769-1774

46. Collins A, Cashman KD, Kiely M. Phyloloquinone (vitamin K1) intakes and serum undercarboxylated osteocalcin levels in Irish postmenopausal women. Br J Nutr 2006;95:982-988

47. McKeown NM, Jacques PF, Gundberg CM, et al. Dietary and nondietary determinats of vitamin K biochemical measures in men and women. J Nutr 2002;132:1329-1334

48. Sogabe N, Tsugawa N, Maruyama R, et al. Nutritional effects of gamma-glutamyl carboxylaze gene polymorphism on the correlation between the vitamin K status and gamma-carboxylation of osteocalcin in young males. J Nutr Sci Vitaminol 2007;53:419-425

49. Yan L, Zhou B, Greenberg D, et al. Vitamin K status of older individuals in northern China is superior to that of older individuals in UK. Br J Nutr 2004;92:939-945

50. Knapen MH, Nieuwenhuijzen-Kruseman AC, Wouters RS, Vermeer C. Correlation of serum osteocalcin fraction with bone mineral density in women during the first 10 years after menopause. Calcif Tissue Int 1998;63:375-379

51. Szulc P, Arlot M, Chapuy MC, et al. Serum under-carboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Mineral Res 1994;9:1591-1595

52. Kanai T, Tagaki T, Masuhiro K, et al. Serum vitamin K level and bone mineral density in post-menopausal women. Int J Gynaecol Obstet 1997;56:25-30

53. Vergnaud P, Garnero P, Meunier PJ et al. Under-carboxylated osteocalcin measured with a specific immnunoassay predicts hip fracture in elderly women: the EPIDOS Study. J Clin Endocrinol Metab 1997;82:719-724

54. Tsugawa N, Shiraki M, Suhara Y, et al. Vitamin K status of healthy Japanese women:age-related vitamin K requirement for γ-carboxylation of osteocalcin. Am J Clin Nutr 2006:83:380-386

55. Braam LA, Knapen MH, Geusens P, et al. Vitamin K1 suplimentation retards bone loss in postmenopausal women between 50 and 60 years of age. Calcif Tissue Int 2003;73:21-26

56. Bolton-Smith C, McMurdo ME, Paterson CR, et al. Two-year randomized controlled trial of vitamin K1 (phylloquinone) and vitamin D3 plus calcium on the bone health of older women J Bone Miner Res 2007;22:509-519

57. Booth SL, Dallal G, Shea MK, et al. Effect of vitamin K supplementation on bone loss in elderly men and women. J Clin Endocrinol Metab 2008;93:1213-1223

58. Volpe SL, Leung MM; Giordano H. Vitamin K supplementation does not significantly impact bone mineral density and biochemical markers of bone in pre- and perimenopausal women. Nutr Res 2008;28:577-582

59. Cockayne S, Adamson J, Lanham-New S, et al. Vitamin K and prevention of fractures. Systematic review and meta-anlysis of randomized controlled trials. Arch Intern Med 2006;1256-1261

60. Knapen MH, Schurgers LJ, Vermeer C. Vitamin K2 supplementation improves hip bone geometry and bone strength indices in postmenopausal women. Osteoporos Int. 2007;18:963-72

61. Iwamoto J, Matsumoto H, Takeda T, et al. Effects of vitamin K2 and risedronate on bone formation and resorption, osteocyte lacunar system, and porosity in the cortical bone of glucocorticoid-treated rats. Calcif Tissue Int 2008;83:121-128

62. Cheung AM, Tile L, Lee Y, et al. Vitamin K supplementation in postmenopausal women with osteopenia (ECKO Trial): a randomized controlled trial. PLoS Med 2008;5:e196

63. Lamson DW, Plaza SM. The anticancer effects of vitamin K. Altern Med Rev 2003;8:301-318

64. Weber P. Vitamin K and bone health. Nutrition 2001;17:880-887

65. Eastell R 2006 Does warfarin increase the risk of osteoporotic fracture? Nat Clin Pract Endocrinol Metab 2006;2:484–485

66. Sugiyama T, Takaki T, Sakanaka K, et al. Warfarin-induced impairment of cortical bone material quality and compensatory adaptation of cortical bone structure to mechanical stimuli J Endocrinol. 2007;194:213-222

67. Avgeri M, Papadopoulou A, Platokouki H, et al. Assessment of bone mineral density and markers of bone turnover in children under long-term oral anticoagulant therapy. J Pediatr Hematol Oncol 2008;30:592-597