Expanding the functional spectrum of vitamin K in bone. Focus on: “Vitamin K promotes mineralization, osteoblast to osteocyte transition, and an anti-catabolic phenotype by γ-carboxylation-dependent and -independent mechanisms”

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VITAMIN K WAS ESTABLISHED as an essential nutrient in 1935 (7) when Danish biochemist Henrik Dam discovered that chicks reared on a diet free of sterols and poor in fat developed large subcutaneous and intramuscular hemorrhages. Almquist and coworkers (1) at the University of California, Berkeley, promptly confirmed the existence of vitamin K, and the Nobel committee recognized the critical importance of this work in 1943 by awarding the physiology and medicine prize to Henrik Dam and Edward Adelbert Doisy for their discoveries (8). For 40 years, research focused on vitamin K’s role in blood coagulation, and its mechanism of action remained undetermined. In 1974, identification of amino-γ-carboxy glutamic acid (Gla) in prothrombin as the product of vitamin K action (29) unequivocally showed that vitamin K is a cofactor for posttranslational carboxylation of Gla residues (24). The Gla residues act as calcium-binding sites that are essential for normal hemostasis (29). Subsequent discoveries of other Gla proteins involved in blood coagulation, such as factors VII, IX, and X synthesized in the liver as well as proteins C, S, and Z (31), gave further insight into vitamin K’s action in hemostasis.

The identification of Gla proteins in the organic matrix of bone boosted research on vitamin K and bone metabolism; these proteins include bone-Gla protein (BGP) (12), matrix-Gla protein (MGP) (25), protein-S (20), Gla-rich protein (GRP) (34), periostin (6), and periostin-like factor (PLF) (26). BGP and MGP have low similarity with blood coagulation factors, although the two categories of molecules are believed to have diverged from a common ancestor (26). BGP, through a posttranslational vitamin K-dependent carboxylation process, gains high affinity for mineral ions and binds to hydroxyapatite. Although a direct role for BGP in the extracellular matrix mineralization process has not been demonstrated in vivo (23), BGP appears to be involved in bone mineral maturation (4) and glucose metabolism (19). BGP has been recently reported to influence β-cell function, insulin sensitivity, adiponectin production, energy expenditure, and adiposity (19, 10), thus assuming the role of a hormone. Because only undercarboxylated BGP is active in glucose metabolism, bone and energy metabolism interaction may rely on crosstalk that evolved from on/off-switch genes (like Esp also known as Ptprv, a gene expressed in osteoblasts and Sertoli cells that encodes a receptor-like protein tyrosine phosphatase) to gain/loss hormonal activity by a vitamin-K-dependent carboxylation/decarboxylation process (15).

MGP, with five vitamin K-dependent Gla residues conferring high affinity for calcium, phosphate, and hydroxyapatite, is a strong inhibitor of calcification and is of recognized importance for vascular health (17). The Gla residues are critical for MGP function, and undercarboxylated, nonactive species of MGP form under inadequate vitamin K status or with vitamin K antagonism. In the intima of atherosclerotic arteries and in Mönckeberg’s sclerosis of the media, where pathological calcifications occur, undercarboxylated MGP is almost exclusively localized in sites of calcification, whereas total and carboxylated MGP are found mostly in the noncalcified areas of the tunica media (27). Given this scenario, impaired γ-carboxylation of the protein at its site of tissue expression may be associated with cardiovascular disease development and progression. Because osteoclasts and osteoblasts also synthesize MGP and MGP accumulates in bone in the carboxylated form with fetuin (27), bone itself may be an important source of calcium-phosphate-fetuin-MGP complexes with a subsequent effect on vascular calcification (5).

GRP, a small Gla protein, localizes to chondrocytes in sturgeon (a fish with an external bony skeleton) and rats and is also expressed in rat bone cells, suggesting a broad role throughout skeletal formation (34). Both osteoblasts and osteocytes yield a clear positive signal for GRP mRNA in trabecular bone. The most remarkable feature of GRP is the high number of Gla residues in its mature form. This enormous potential for calcium binding suggests its role as a physiological calcium modulator in the extracellular domain (34). In fact, a partition system without contributions from the bone remodeling system controls basal level and minute-by-minute correction of plasma Ca²⁺ by outward and inward Ca²⁺ fluxes from and into an exchangeable ionic pool in the bone endocanalicular network (21). The biomolecular mechanisms underlying this critical process in calcium homeostasis are unknown, but the osteocytes bone lining cells syncytium may modulate it through pump-leak mechanisms (21) and/or by the production of noncollagenous proteins (33). Because they complex large amounts of calcium and are physically bound to hydroxyapatite, Gla proteins produced by bone cells may establish an appropriate free calcium concentration in the extracellular fluid to support life (33). In particular, GRP appears to meet the physicochemical requirements for such a demanding role, being produced by osteocytes and having a unique ability to bind calcium that appears to have been conserved over more than 450 million years of evolution (34). This Gla protein thus broadens the functional spectrum of the vitamin K-dependent carboxylation/decarboxylation process in bone to calcium homeostasis.
Finally, periostin (6) and PLF (26) are novel γ-carboxylated proteins. Periostin is highly expressed in bone extracellular matrix and is produced by mesenchymal stromal cells (6), a population of adherent cells that give rise to all nonhematopoietic cell lineages in the bone (stromal fibroblasts, osteocytes, chondrocytes, and adipocytes) and that support hematopoiesis (9). PLF, one of the naturally occurring splice variants from the periostin locus, is primarily expressed in the perios- teum during the early adaptive stage of remodeling (26). Periostin interacts via its fasciclin domains with integrins on the cell surface and with extracellular matrix proteins. Intrinsic to these interactions are the evolving functions that periostin plays as a factor necessary for tissue development, maturation, and repair. The γ-carboxylation of periostin in nonmineralized tissues expands the role of the vitamin K-dependent carboxylation/decarboxylation process to angiogenesis, myocardial remodeling after infarction, and tumor metastasis (6).

The role of protein-S in bone remains partially undefined (20), but these specific features of BGP, MGP, GRP, periostin, and PLF have suggested that tissue- and cell-specific vitamin K-dependent carboxylation/decarboxylation processes have served as evolutionary adaptations, establishing regulatory pathways in bone that contribute to fine tuning of bone metabolism and of energy metabolism, calcification, plasma calcium homeostasis, and angiogenesis. The study of Atkins et al. (2) further expands the functional spectrum of vitamin K in bone. They provide compelling evidence that synthetic vitamin K and homologues induce bone cellular events by both vitamin K activation-dependent and independent mechanisms. In their well-designed study, Atkins and coworkers (2) demonstrate that vitamin K homologues induce bone matrix mineralization, promote osteoblast transition into osteocytes, and inhibit expression of RANKL (receptor activator of nuclear factor-κB ligand) in the osteocyte cell-like line MLO-Y4, thus potentially hampering osteoclastogenesis. The RANKL:osteoprotegerin equilibrium potentially achieved under vitamin K stimulus might determine a positive bone mass outcome by relatively reducing the resorption phase of the activated bone remodeling sequence. This vitamin K inhibitory effect on RANKL production deserves further exploration, however, being in agreement with results observed in vivo (22) but in disagreement with those observed in vitro in a more immature osteoblast phenotype (16).

The positive effect of vitamin K exposure on matrix mineralization is partly due to γ-carboxylation; in fact warfarin, a known binder of the vitamin K epoxide reductase that subsequently inhibits vitamin K recycling, interferes with these positive effects. As discussed by Atkins et al. (2), Gla proteins expressed in bone should, therefore, mediate the promineralization effect; these proteins include BGP, MGP, protein-S, periostin, and PLF, which, as described, display calcium-binding potential when vitamin K γ-carboxylates their Gla residues. On the other hand, Atkins et al. (2) found that warfarin had no influence on vitamin K-dependent effects on osteoblast-to-osteocyte transition, enhanced expression of osteocyte marker E11, or the shift of RANKL:osteoprotegerin towards an anti-osteoclastogenic environment. This warfarin resistance points to a vitamin K pathway in bone that is γ-carboxylation independent and potentially related to its genomic action. Of note, MK-4 (K2) arises from tissue-specific conversion of dietary K1 (3), is produced in osteoblast (30), and differs structurally from K1 in the configuration of its side chain; it was originally recognized as a bone-resorption inhibitory factor through γ-carboxylation-independent mechanisms possibly activated by its side chain (11).

The observation that vitamins K2 and K3 have a transcriptional regulatory function in osteoblasts in addition to their role as enzyme cofactors challenges the concept that vitamin K in bone acts solely by a tissue- and cell-specific carboxylation/ decarboxylation process. This is in accordance with the identification of a novel signaling pathway of vitamin K action in bone through a transcription factor, the steroid, and xenobiotic receptor (SXR) (32). SXR belongs to the nuclear receptor subfamily-1 that binds to specific SXR response elements in the genome (SXRE), regulating target gene transcription. Vitamin K2 (MK-4) acts as a SXR ligand and upregulates expression of the prototypical SXR target gene cytochrome P450 3A4 and bone marker genes such as alkaline phosphatase and osteoprotegerin (32). SXR-dependent vitamin K2 target genes participating in extracellular matrix formation and collagen accumulation in osteoblastic cells were thus identified (13). Further dissection of the vitamin K activation pathways also led to the discovery in osteoblastic cells of target genes (GDF15 and STC2) upregulated by the vitamin K metabolite MK-4 in a SXR-independent manner (14).

The Atkins et al. study (2) describes two important outcomes related to the potential use of vitamin K in bone health, ideas that remain widely debated (3). First, it sustains the view that the vitamin K regulatory pathway in bone does not exclusively involve tissue- and cell-specific vitamin K-dependent carboxylation/decarboxylation processes. Second, it has shifted the focus regarding vitamin K’s therapeutic potential in bone from its as-yet-unproven effects on bone quantity (3) to its potential effects on bone quality. By demonstrating vitamin K’s ability to promote matrix mineralization and osteocyte differentiation, Atkins et al. (2) provide compelling evidence that vitamin K

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Fig. 1. Simplified depiction of the pleiotropic function of vitamin K in bone. Vitamin K acts by a tissue- and cell-specific carboxylation/decarboxylation process of amino-γ-carboxy glutamic acid (Gla) proteins that are endowed of structural and regulatory functions in bone and in nonmineralized tissues and by a novel signaling pathway through a transcription factor, the steroid and xenobiotic receptor (SXR). The vitamin K’s ability to promote collagen accumulation, cell-matrix interactions, matrix mineralization, mineral matura-

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modulates structural parameters and cell effectors of bone strength. Matrix mineralization and optimal osteocyte density are indeed constitutive factors of the mechanical competence of bone (28) (Fig. 1).

REFERENCES