Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1543–1551

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Mevalonate-suppressive dietary isoprenoids for bone health

Huanbiao Mo[a,*], Hoda Yeganehjoo[a], Anureet Shah[a], Warren K. Mo[b], Ima Nirwana Soelaiman[c], Chwan-Li Shen[d]

[a]Department of Nutrition and Food Sciences, Texas Woman's University, Denton, TX 76204, USA
[b]Texas Academy of Mathematics and Science, University of North Texas, Denton, TX 76203, USA
[c]Department of Pharmacology, National University of Malaysia (UKM), Kuala Lumpur, Malaysia
[d]Department of Pathology, Texas Tech University Health Sciences Center, BB 198, Lubbock, TX 79430-9097, USA

Received 12 June 2012; received in revised form 13 July 2012; accepted 19 July 2012

Abstract

Osteoclastogenesis and osteoblastogenesis, the balancing acts for optimal bone health, are under the regulation of small guanosine triphosphate-binding proteins (GTPases) including Ras, Rac, Rho and Rab. The activities of GTPases require post-translational modification with mevalonate-derived prenyl pyrophosphates. Mevalonate deprivation induced by competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (e.g., statins) prevents the activation of GTPases, suppresses the expression of the receptor for activation of nuclear factor kappa B (NFκB) ligand (RANKL) and activation of NFκB and, consequently, inhibits osteoclast differentiation and induces osteoclast apoptosis. In contrast, statin-mediated inactivation of GTPases enhances alkaline phosphatase activity and the expression of bone morphogenetic protein-2, vascular epithelial growth factor, and osteocalcin in osteoblasts and induces osteoblast proliferation and differentiation. Animal studies show that statins inhibit bone resorption and increase bone formation. The anabolic effect of statins and other mevalonate pathway-suppressive pharmaceuticals resembles the anti-osteoclastogenic and bone-protective activities conferred by dietary isoprenoids, secondary products of plant mevalonate metabolism. The tocotrienols, vitamin E molecules with HMG CoA reductase-suppressive activity, induce mevalonate deprivation and concomitantly suppress the expression of RANKL and cyclooxygenase-2, the production of prostaglandin E2 and the activation of NFκB. Accordingly, tocotrienols inhibit osteoclast differentiation and induce osteoclast apoptosis, impacts reminiscent of those of statins. In vivo studies confirm the bone protective activity of tocotrienols at nontoxic doses. Blends of tocotrienols, statins and isoprenoids widely found in fruits, vegetables, grains, herbs, spices, and essential oils may synergistically suppress osteoclastogenesis while promoting osteoblastogenesis, offering a novel approach to bone health that warrants clinical studies.

Keywords: Mevalonate; Isoprenoid; Tocotrienol; HMG CoA reductase; Osteoclast; Osteoblast

Optimal bone health requires a balance between osteoblastic and osteoclastic activities, a balance that tips towards osteoclastic activity with age. The differentiation of osteoclasts and osteoblasts is regulated by small guanosine triphosphate-binding proteins (GTPases) that depend on the mevalonate-derived intermediates for their post-translational modification and biological activities. We first delineate the role of mevalonate pathway in the differentiation of osteoclasts and osteoblasts. Drawing evidence from in vitro, in vivo and clinical studies with pharmaceuticals including the statins, bisphosphonates, prenyl transferase inhibitors and menaquinone derivatives, we propose that suppression of the mevalonate pathway activities is a valid approach to bone protection. We then present our central thesis that dietary isoprenoids — particularly the tocotrienol isomers of vitamin E with mevalonate-suppressive activities — and their synergistic interactions may have potential for bone health. We limit the scope of our review to studies with agents that suppress mevalonate pathway activities.

1. Regulation of bone formation — osteoblastogenesis and osteoclastogenesis

Human bone is a dynamic organ maintained and reconstructed by bone multicellular units composed of osteoblasts and osteoclasts, two main types of cells that are involved in bone modeling and remodeling [1]. Bone integrity depends on the balancing act of

Abbreviations: ALP, alkaline phosphatase; BMD, bone mineral density; BMP, bone morphogenetic protein; COX-2, cyclooxygenase-2; FTI, farnesyl transferase inhibitor; GGTI, geranylgeranyl transferase inhibitor; GTPase, small guanosine triphosphate-binding protein; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LPS, lipopolysaccharide; NFκB, nuclear factor kappa B; OPG, osteoprotegerin; PGE2, prostaglandin E2; RANKL, receptor for activation of nuclear factor kappa B (NFκB) ligand; VEGF, vascular epithelial growth factor.

* Corresponding author. Tel.: +1 940 898 2712; fax: +1 940 898 2634.
E-mail address: hmo@twu.edu (H. Mo).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2012.07.007

osteoclast-mediated bone resorption and osteoblast-mediated bone synthesis [2–4]. When bone resorption exceeds bone formation, deteriorated bone health including bone loss, decreased bone mineral density and structural damage may occur [3]. Estrogen deficiency, for example, increases the rate of bone remodeling, prolonging the life span of osteoclasts whereas shortening that of osteoblasts, resulting in net bone loss that is common after menopause.

2. The role of mevalonate in the differentiation of osteoclasts and osteoblasts

The important roles of osteoclasts and osteoblasts necessitate fine regulatory mechanisms for the differentiation and formation of these cells. The differentiation of monocytic cells to osteoclasts requires the receptor for activation of nuclear factor kappa B (NFκB) ligand (RANKL) and NFκB[3,4], and osteoclast survival, differentiation and function require the GTPases including Ras, Rac [5–7], Rho and Rab [8–10] (Fig. 1). The membrane attachment and biological activity of these small GTPases require prenylation, i.e., post-translational modification with mevalonate-derived intermediates, namely farnesyl- and geranylgeranyl-pyrophosphates [11]. Ras involved in cell proliferation and survival is farnesylated [12] whereas Rho, Rac and Rab responsible for actin-cytoskeletal dynamics, cell adhesion and motility are geranylgeranylated [13]. Loss of prenylation of Rho, Rac and Rab [8] leads to osteoclast apoptosis [14,15] and loss of the ruffled border, a convoluted region of plasma membrane formed between the osteoclasts and the bone surface that is essential for the resorption process [3].

The differentiation of osteoblasts from mesenchymal stem cells, differing from that of osteoclasts, is controlled by growth factors such as the bone morphogenetic proteins (BMPs) [2,15], a class of six proteins including the autocrine factor BMP-2 that promote osteoblast proliferation and differentiation [16,17], and vascular epithelial growth factor (VEGF) that stimulates osteoblast differentiation [18]. BMP-2 stimulates the differentiation of mesenchymal cells into osteoblasts and chondrocytes by binding to its receptor, a Ser/Thr kinase, and then activating Smad 1 and Smad 5, which in turn induce Cbfa1 (Runx2) that stimulates protein expression for bone formation [19]. Prenylation of Rho and Ras block BMP-2 expression [20] and osteoblast differentiation [21,22] (Fig. 1).

The contrasting roles of protein prenylation in promoting osteoclast differentiation and suppressing osteoblast differentiation may underlie the findings that the mevalonate pathway suppressors inhibit the osteoclast differentiation while stimulating osteoblast differentiation. The mevalonate suppressors including pharmaceuticals and dietary factors either control the pool of mevalonate-derived products for the prenylation of the small GTPases (statins, bisphosphonates, menaquinone derivatives, and isoprenoids) or block the transfer of prenyl groups to GTPases (prenyl transferase inhibitors and bisphosphonates) (Fig. 1). Preclinical mechanistic studies of mevalonate-suppressive pharmaceuticals presented the mevalonate pathway as a viable target for improving bone health and paved the way for nutritional intervention with dietary factors, particularly the mevalonate-suppressive isoprenoids.

3. The impact of mevalonate-suppressive pharmaceuticals

3.1. Statins: in vitro, in vivo and clinical studies

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase is the rate-limiting activity of the mevalonate pathway [11]. Preclinical studies with the statins, competitive inhibitors of HMG CoA reductase, offered a glimpse of the potential application of mevalonate suppressors. The statins inhibit bone resorption [6,23,24] by inhibiting differentiation [6,25–28] and inducing apoptosis in osteoclasts [5,6] while enhancing osteoblast differentiation [16,29–35] (Table 1).

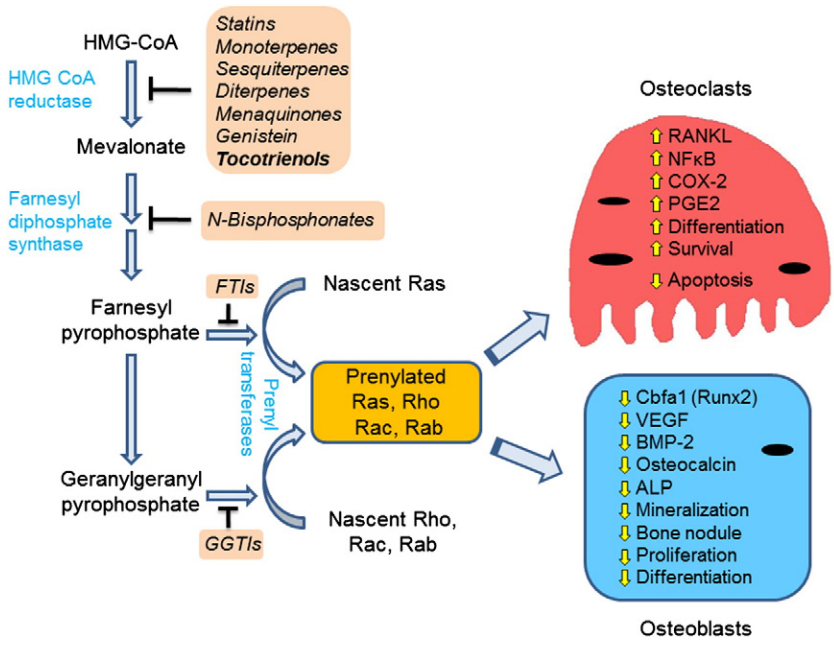

Fig. 1. The proposed mechanism of action for mevalonate suppressors in osteoclasts and osteoblasts. The mevalonate pathway provides essential intermediates, farnesyl- and geranylgeranyl pyrophosphates, for the post-translational prenylation of GTPases including Ras, Rho, Rac and Rab. Prenylated GTPases have dichotomous effects on osteoclasts and osteoblasts. They up-regulate or activate RANKL, NFκB, COX-2, and PGE2, support the survival and differentiation of osteoclasts, and suppress apoptosis of osteoclasts while down-regulating Cbfa1 (Runx2), VEGF, BMP-2, osteocalcin, ALP, mineralization, bone nodule formation, and the proliferation and differentiation of osteoblasts (yellow arrows). The suppressors of HMG CoA reductase (statins, monoterpenes, sesquiterpenes, diterpenes, menaquinones, genistein and tocotrienols), inhibitors of farnesyl diphosphate synthase (bisphosphonates), and prenyltransferase inhibitors [GGTIs and farnesyl transferase inhibitor (FTIs)] increase bone mineral density as a consequence of suppressing the effects of prenylated GTPases on osteoclast and osteoblast activities.

Table 1
In vitro studies showing the impact of mevalonate-suppressive pharmaceuticals and isoprenoids on osteoclasts and osteoblasts

Impact	Pharmaceuticals	Isoprenoids
Inhibit osteoclast differentiation	Statin [6,25–28]*	Tocotrienol [36]
	GGTI [28,37]	Menatetrenone [38]
	Bisphosphonate [6,37]	Geranylgeraniol [38,39]
		Borneol [39]
		Menthol [39]
		T-verbenol [39]
		Perillyl alcohol [39]
		Farnesol [39]
		Zerumbone [40]
Increase OPG expression	Statin [41]	
	Bisphosphonate [41]	
Suppress RANKL expression	Statin [41]	Tocotrienols [36]
	Bisphosphonate [41]	Geranylgeraniol [38]
Induce osteoclast apoptosis	Statin [5,6]	
	Bisphosphonate [5,37,42,43]	
	GGTI [37]	
Suppress NFκB activation	Statin [26,44]	Tocotrienol [36,45,46]
	FTI [47]	Zerumbone [40]
Suppress COX-2 and PGE2 levels		Tocotrienol [45,48]
		Menatetrenone [38]
Induce osteoblast proliferation	Statin [32]	
Induce osteoblast differentiation	Statin [16,29–35]	
	GGTI [49]	
Enhance Cbfa1 (Runx2) expression	Statin [50]	
Enhance BMP-2 expression	Statin [16,29,31,33,35,51–58]	
Enhance VEGF expression	Statin [33,57]	
Enhance osteocalcin expression	Statin [29,33,35,50,51,58,59]	
Enhance ALP activity in osteoblasts	Statin [29,32,33,35,59,60]	Borneol [39]
		Menthol [39]
Suppress prenylation of Ras, Rho, Rac, Rab	Statin [16,41,51,57]	Tocotrienol [61,62]
	Bisphosphonate [5,8,37,41,42]	
	GGTI [28]	
	Tocotrienol analog [62]	
Suppress HMG CoA reductase	Statin [11,16]	Tocotrienol [63–66]
	Bisphosphonate [67]	Farnesol [68,69]
		Geranylgeraniol [70–73]
		Borneol [74]
		Menthol [74,75]
		Perillyl alcohol [76]
Inhibit prenyl transferase activity	Bisphosphonate [77]	
	GGTI [37]	
	FTI [47]	

* Reference numbers.

The ability of forty statin analogs to inhibit HMG CoA reductase is positively correlated with their ability to inhibit bone resorption [23]. The effects of statins on osteoclasts are attributed to the inhibition of protein prenylation [27,78] and, consequently, abrogation of RANKL-induced NFκB activation [26,44], effects reversible with supplemental geranylgeranyl pyrophosphate [28] and geranylgeraniol [39,78]; the latter upon phosphorylation [79,80] forms the substrate for prenylation. Conversely, statins suppress Rho protein prenylation [16,51] and signaling [51] and increase the expression of Cbfa1 (Runx2) [50], BMP-2 [16,29,31,33,35,51–58] and VEGF [33,57] in osteoblastic cells with a concomitant increase in the expression of osteocalcin [29,33,35,50,51,58,59], a late marker for osteoblasts [81]. Mevalonate or geranylgeranyl pyrophosphate negates the effect of statins on the expression of BMP-2 [31,51,56] and VEGF [57], suggesting the statin effect is directly related to suppression of HMG CoA reductase activity [23]. Statin-containing Chinese red yeast rice also stimulated the proliferation, alkaline phosphatase (ALP) activity and differentiation of MC3T3-E1 osteoblast-like cells [32].

The anabolic effect of statins shown in the studies of bone cells and organ [31] is further supported by animal studies (Table 2). Statins increased the number of mature osteoblasts, bone formation and bone volume [31,54], helped bone repair [89], prevented the steroid-mediated bone loss [90] and reduced the ovariectomy-induced increase in markers of bone metabolism [24]. Chinese red yeast rice also stimulated new bone formation in New Zealand white rabbits, an effect accompanied by increased cell viability and ALP activity in rat UMR 106 osteoblastic cells [60].

These preclinical findings are consistent with most [53,93–104], but not all [105–107], case–control studies where statin use is associated with increased bone mineral density and reduced risk of fracture. The degree of reduction in hip fracture is related to the extent of statin use whereas non-statin lipid-lowering agents are

Table 2
In vivo and human studies showing the impact of statins and isoprenoids on bone

Impact	Statins	Isoprenoids
In vivo		
Inhibit bone resorption	[6,23,24]*	Tocotrienol [82,83]
		Menatetrenone [84]
		Camphor [85]
		Eucalyptol [85]
		Borneol [85]
		Bornylacetate [85]
		Thymol [85]
		Menthol [85]
		Thujone [85]
		A-pinene [85]
		B-pinene [85]
		Zerumbone [40]
Increase bone growth and formation	[16,31,54,60,86,87]	Tocotrienol [82,88]
Help bone repair	[86,89]	
Prevent steroid-mediated bone loss	[90]	
Inhibit osteoclast differentiation & activity	[86]	Monoterpenes mixture [85]
Increase number of mature osteoblasts	[31]	Tocotrienol [82]
Increase ALP activity in osteoblasts	[86]	
Increase BMP-2 expression	[86]	
Inhibit RANKL expression	[86]	
Increase serum osteocalcin level		tocotrienol [82]
Increase bone calcification		tocotrienol [91,92]
Case–control		
Associated with higher BMD & reduced risk of fracture	[53,93–104]	
Not associated with BMD or fracture risk	[105–107]	
Prospective cohort		
Inhibit bone resorption	[101]	
Increase BMD	[99,108,109]	
Reduce risk of fracture	[110]	
Increase serum osteocalcin	[111]	
Not associated with BMD or fracture risk	[103,112]	
Cross-sectional		
Increase BMD and reduce fracture risk	[113]	
Randomized trials		
Maintain peripheral cortical bone density		Menatetrenone [114]
Mixed effects on BMD	[115]	
Age-dependent increase in osteocalcin	[116]	
Not associated with BMD or fracture risk	[103,110,117–120]	

* Reference numbers.

ineffective [95], suggesting the essential role of HMG CoA reductase inhibition. Most [99,101,108–111,113], but not all [103,112], prospective and cross-sectional studies also support statin-mediated bone protection. Randomized clinical trials, however, have failed to prove the efficacy of statins [103,110,117–120]. The discrepancy in these studies may be attributed to the varying degrees of hydrophobicity of the diverse statins, limited systemic distribution of statins [31,121] and low doses of statins used in these studies with cholesterol-lowering as the end point [53].

3.2. Bisphosphonates, prenyl transferase inhibitors, and menaquinone derivatives

Consistent with the bone-protection offered by the statins, a second class of mevalonate pathway suppressors, the nitrogen-containing bisphosphonates, induce osteoclast apoptosis [5,37,42,43]. The bisphosphonates inhibit farnesyl diphosphate synthase [6,7,42,43,122–124], the activity leading to the syntheses of farnesyl- and geranylgeranyl- pyrophosphates (Fig. 1), and suppress the prenylation and membrane association of Ras [5,42], Rho, Rac and Rab [8]. The bisphosphonate effects were attenuated by supplemental farnesol [125] and geranylgeraniol [6,37,43,125], suggesting that protein prenylation plays a role in bisphosphonate-mediated osteoclast apoptosis. Other bisphosphonates inhibit prenyl transferase activity [77] that catalyzes the prenylation and as a secondary function, suppress the expression of HMG CoA reductase in osteoclasts [67]. "Inappropriate" stimulation of signaling pathways initiated by unprenylated GTPases might have contributed to the effects of bisphosphonates [126].

Parallel to the bisphosphonate-mediated osteoclast apoptosis is the finding that a geranylgeranyl transferase inhibitor (GGTI) (Fig. 1) disrupts the osteoclast cytoskeleton, induces apoptosis in isolated osteoclasts, prevents osteoclast formation, and inhibits bone resorption [28,37]. GGTIs also increased osteoblastogenesis [49]. Menatetrenone, a homologue of vitamin K2 or menaquinone (Fig. 1), and its geranylgeraniol side chain with HMG CoA reductase-suppressive activity [70–73], inhibit 1,25$(OH)_2$vitamin D_3-induced osteoclast-like multinucleated cell formation and 1,25$(OH)_2$vitamin D_3- and prostaglandin E_2 (PGE2)-induced bone resorption; suppression of RANKL expression may mediate the effect of geranylgeraniol [38]. In contrast, vitamin K1 or phylloquinone, and its phytol side chain with no HMG CoA reductase-suppressive activity, offer no such protection [127–129]. The anti-resorption activity of menatetrenone was manifested in ovariectomized rats [84]. In osteoporotic patients, menatetrenone at 90 mg/day proved safe and effective in maintaining peripheral cortical bone density [114].

4. The impact of mevalonate-suppressive isoprenoids

The bone protection afforded by the aforementioned mevalonate-suppressive pharmaceuticals, the statins, bisphosphonates, prenyl transferase inhibitors, and menatetrenone suggests that mevalonate-suppressive dietary constituents may also modulate the osteoclastic and osteoblastic activities. Many of the estimated 23,000 secondary products of plant mevalonate pathway, namely, isoprenoids [130], that are ubiquitous in fruits, vegetables and other plant foods have been shown to suppress HMG CoA reductase [131,132]. Literature has recorded bone protective actions of the "pure" isoprenoids, mainly the mono-, sesqui- and di-terpenes of the isoprenoid family consisting only of multiples of the five-carbon isoprene unit [131]. The 10-carbon monoterpenes composed of two isoprene units are the main constituents of essential oils and are widely distributed in the plant kingdom. In addition to the aforementioned diterpene geranylgeraniol, mevalonate-suppressive monoterpenes (Fig. 1) [74–76,132–135] including borneol, menthol, *t*-verbenol, perillyl alcohol and perillic acid at physiologically attainable levels (1–100 μmol/L) and levels nontoxic to osteoblasts inhibit the formation of osteoclasts and that of their actin ring [39], an indication of cell polarization and a characteristic of resorbing osteoclasts. Geranylgeraniol and a sesquiterpene farnesol potentiated the anti-osteoclastogenic effect of menthol and perillyl alcohol. Borneol and menthol also induced ALP expression in osteoblasts [39].

In a separate study, dietary essential oils of pine, dwarf pine, eucalyptus, sage, juniper, rosemary and thyme, in descending order of potency, inhibited bone resorption in rats [85]. The nine monoterpene constituents of these oils, namely camphor, eucalyptol, borneol, bornylacetate, thymol, menthol, thujone, α-pinene and β-pinene, when fed in diet individually and in a blend, showed anti-resorption activity as well. These results are consistent with our earlier finding of the additive impact of monoterpenes on the mevalonate pathway [136]. Dietary pine oil also reduced trabecular bone mineral density (BMD) loss in aged ovariectomized rats [85]. The resorptive activity of osteoclasts as measured by number of resorption pits per osteoclast was suppressed by thymol, borneol, camphor and *cis*-verbenol, a metabolite of α-pinene. Borneol also suppressed the formation of actin ring in osteoclasts. Zerumbone, a sesquiterpene, inhibited RANKL- and tumor cell-induced osteoclastogenesis and reduced breast cancer MDA-MB-231-induced bone loss in mice [40]. Noteworthy is that the isoflavone genistein (Fig. 1), an inhibitor of osteoclast-like cell formation [137–139], also inhibits HMG CoA reductase activity [140,141]. These actions of isoprenoids resonate with the association between intake of fruits and vegetables and increased bone mineral density [142–146] and reduced risk of bone fracture [142,144].

5. Tocotrienols — the potent mevalonate suppressors with bone protective benefits

5.1. Tocotrienols down-regulate HMG CoA reductase

Among the most potent mevalonate-suppressive dietary constituents are the tocotrienols (Fig. 1) [63,64,147], vitamin E isomers with an unsaturated farnesol side chain [148,149]. The tocotrienols are "mixed" isoprenoids with their farnesyl moiety derived via the mevalonate pathway [131]. The tocotrienols have a wide presence in plant foods including avocados, bananas, berries, cabbage, cherries, coconut, corn, Kiwi, green pea, onions, peaches, pears, plums [150–152], grape [153], peanuts [154], hazelnut [155], horse chestnuts, litchi [156], cereals, wheat [157] and olive [158]. Specialty oils from palm, rice bran, barley and oat are good sources of γ-tocotrienol [159] while annatto has emerged as a viable commercial source of δ-tocotrienol [160].

The tocotrienol-mediated down-regulation of reductase mimics that triggered by farnesol, the endogenous secondary modulator of reductase [68,69]. The initial finding of the hypocholesterolemic effect of barley flour [161], barley extract [162] and one of the constituents, α-tocotrienol, via suppression of HMG CoA reductase [65] led to subsequent efforts delineating the tocotrienol-mediated post-transcriptional down-regulation of HMG CoA reductase [63,64,66]. Additionally, δ-tocotrienol was found to down-regulate HMG CoA reductase at the transcriptional level [64]. In contrast, tocopherols, the vitamin E molecules with a saturated phytol tail, did not suppress HMG CoA reductase [66]. In fact, α-tocopherol was found to attenuate the impact of tocotrienols on HMG CoA reductase [163] or even induce HMG CoA reductase activity [164].

5.2. Tocotrienols: in vitro and in vivo studies

The HMG CoA reductase-suppressive activity of tocotrienols may have afforded tocotrienols bone-protective properties. Tocotrienols

inhibited lipopolysaccharide (LPS)-induced expression of cyclooxygenase-2 (COX-2), PGE2, interleukin-6 and tumor necrosis factor α in murine RAW264.7 macrophages [48]. Similarly, tocotrienol-rich fractions inhibited LPS-induced inducible nitric oxide synthase, COX-2 and NFκB expression in human monocytic cells [45]. This anti-osteoclastogenic activity of tocotrienols was shown at concentrations well below that shown to be toxic in primary osteoblasts (IC_{50}=290 μmol/L)[165]. α-Tocotrienol, but not α-tocopherol, inhibited osteoclastogenesis in coculture of osteoblasts and bone marrow cells, reduced RANKL expression in osteoblasts, blocked RANKL-induced osteoclast differentiation and the activation of extracellular signal-regulated kinases and NFκB and suppressed bone resorbing activity of mature osteoclasts [36]. Tocotrienols [61,62] and analogs [62] have been shown to suppress the prenylation of Ras protein and consequently reduce the level of Ras protein due to the higher turnover rate of unprenylated Ras in human lung and melanoma cells. It remains unknown whether such effect exists in osteoclasts.

Animal studies also suggest that tocotrienols offer bone protection. Tocotrienol mixture at 60- and 100-mg/kg body weight restored free radical-induced reduction in serum level of osteocalcin, the number of osteoblasts, and bone formation in male Wistar rats while reducing the level of bone-resorbing cytokines [82]. Oral intake of γ-tocotrienol [91,166] and a mixture of tocotrienols [91,92] increased bone calcification in Sprague–Dawley rats. A tocotrienol-enhanced fraction and γ-tocotrienol also counteracted the nicotine effect on bone resorption in rats [83]. Oral gavage of tocotrienols at 60 mg/kg improved the static (osteoclast and osteoblast numbers, eroded surface/bone surface ratio, osteoid surface/bone surface ratio, osteoid volume/bone volume ratio) and dynamic (single-labeled surface/bone surface ratio, double-labeled/bone surface ratio, mineralized surface/bone surface ratio, mineral apposition rate, bone formation rate/bone surface ratio) parameters of bone following four months of treatment in Sprague–Dawley male rats [167]. Toxicity of mixed tocotrienols including bleeding tendency and renal impairment was observed at 500 mg/kg body weight. No toxicity was observed at levels up to 200 mg/kg body weight [168] that is well above the aforementioned effective doses.

5.3. The differential impacts of tocotrienols and tocopherols

In all the comparison studies, α-tocopherol [91,92,166,169], tocopherols [169] and tocopheryl esters [82,91] with no mevalonate suppressive activity [63] offer either no protection or less-than-tocotrienol effects [83,170,171]. γ-Tocotrienol at 60 mg/kg decreased trabecular separation and increased trabecular bone volume and thickness, trabecular number, load value, displacement, stress value, strain value, viscoelasticity and stiffness in male rats to significantly greater extent than did equal dose of α-tocopherol [88]. An earlier study showed no beneficial effects of mixed tocopherols when fed to orchidectomized rats at a much higher level (656 mg/kg diet) [169]. In fact, tocopherols may have detrimental effects. α-Tocopherol stimulated osteoclast fusion independent of its antioxidant activity. Ttpa−/− mice with α-tocopherol transfer protein deficiency and hence lower systemic circulation of α-tocopherol had high bone mass, while wild-type mice or rats fed α-tocopherol supplementation at levels comparable to that for human consumption lost bone mass [172].

The differential impacts of tocotrienols and tocopherols are reminiscent of those of menaquinones and phylloquinones. The tocotrienols and menaquinones contain a mevalonate-suppressive isoprenyl side chain, whereas the phytol moiety of tocopherols and phylloquinones has no impact on HMG CoA reductase. These in vitro and in vivo studies, albeit suggestive of tocotrienol-mediated mevalonate deprivation, did not prove whether the bone-protective effect of tocotrienols is directly mediated by suppression of HMG CoA reductase activity. It should be noted that the aforementioned mevalonate-suppressive agents, the statins [26,44], prenyltransferase inhibitors [47], monoterpenes [173], zerumbone [40] and the tocotrienols [36,45,46,174,175], but not α-tocopherol [176], inhibit the NFκB activity that is critical in osteoclastogenesis. The reversal of the effect of tocotrienols on NFκB by supplemental mevalonate [176] lends additional support to the role of HMG CoA reductase in tocotrienol-mediated bone protection.

6. Summary and future directions

The mevalonate-suppressive pharmaceuticals including the statins, bisphosphonates, prenyl transferase inhibitors and menaquinone derivatives inhibit osteoclastogenesis by limiting the prenylation of GTPases and consequently, down-regulating OPG, RANKL and NFκB. In contrast, these agents promote osteoblastogenesis by up-regulating BMP-2, VEGF, osteocalcin, Cbfa1 (Runx2) and ALP activity. Dietary isoprenoids, e.g., mono-, sesqui- and di-terpenes and the more potent tocotrienols, down-regulate HMG CoA reductase and suppress the expression of PGE2 and the activities of COX-2 and NFκB. The signaling pathways that mediate the effect of prenylated GTPases on these biomarker proteins of osteoclastogenesis and osteoblastogenesis remain unknown and warrant further investigation. Bioavailability and pharmacokinetics of tocotrienols, particularly those applied to bone, remain unexplored. Clinical studies are needed to verify the efficacy of tocotrienols in bone protection. Tocotrienols potentiate the statin-mediated suppression of reductase [134,147,177–179]. Synergy could be obtained by combining tocotrienols, down-regulators of HMG CoA reductase [63–66,147], with the statins, the competitive inhibitors of reductase [132], effectively lowering the required doses of both agents. Synergistic effect [73,179–181] may also be attainable with tocotrienols and diverse isoprenoids [136] widely distributed in fruits, vegetables, herbs, spices and essential oils [131]. Further investigations are needed to elucidate the underlying mechanisms for the tocotrienol-mediated bone protection. Nevertheless, suppression of mevalonate pathway activities, evidenced by studies with pharmaceuticals and dietary isoprenoids, proved to be a promising approach in promoting bone health. Bone protective activity of tocotrienols would add to the cancer chemopreventive [148], hypocholesterolemic [182], cardioprotective [183] and neuroprotective [184] activities of this group of orally available, convenient and safe dietary ingredients.

Acknowledgments

This work was partially supported by the Agriculture and Food Research Initiative Grant 2009–02941 from the USDA National Institute for Food and Agriculture, Texas Department of Agriculture Food and Fiber Research Program, and Texas Woman's University Research Enhancement Program.

References

[1] Seeman E, Delmas PD. Bone quality — the material and structural basis of bone strength and fragility. N Engl J Med 2006;354:2250-61.
[2] Ducy P, Schinke T, Karsenty G. The osteoblast: a sophisticated fibroblast under central surveillance. Science 2000;289:1501-4.
[3] Teitelbaum SL. Bone resorption by osteoclasts. Science 2000;289:1504-8.
[4] Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337-42.
[5] Luckman SP, Hughes DE, Coxon FP, Graham R, Russell G, Rogers MJ. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. J Bone Miner Res 1998;13:581-9.
[6] Fisher JE, Rogers MJ, Halasy JM, Luckman SP, Hughes DE, Masarachia PJ, et al. Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA 1999;96:133-8.

[7] van Beek E, Pieterman E, Cohen L, Lowik C, Papapoulos S. Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. Biochem Biophys Res Commun 1999;264:108-11.
[8] Coxon FP, Ebetino FH, Mules EH, Seabra MC, McKenna CE, Rogers MJ. Phosphonocarboxylate inhibitors of Rab geranylgeranyl transferase disrupt the prenylation and membrane localization of Rab proteins in osteoclasts in vitro and in vivo. Bone 2005;37:349-58.
[9] Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 1996;65:241-69.
[10] Reid IR. Anti-resorptive therapies for osteoporosis. Semin Cell Dev Biol 2008;19:473-8.
[11] Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature 1990;343:425-30.
[12] Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. J Natl Cancer Inst 2001;93:1062-74.
[13] Andela VB, Pirri M, Schwarz EM, Puzas EJ, O'Keefe RJ, Rosenblatt JD, et al. The mevalonate synthesis pathway as a therapeutic target in cancer. Clin Orthop 2003:S59-66.
[14] Rogers MJ. New insights into the molecular mechanisms of action of bisphosphonates. Curr Pharm Des 2003;9:2643-58.
[15] Rodan GA, Martin TJ. Therapeutic approaches to bone diseases. Science 2000;289:1508-14.
[16] Garrett IR, Gutierrez G, Mundy GR. Statins and bone formation. Curr Pharm Des 2001;7:715-36.
[17] Harris SE, Bonewald LF, Harris MA, Sabatini M, Dallas S, Feng JQ, et al. Effects of transforming growth factor β on bone nodule formation and expression of bone morphogenetic protein 2, osteocalcin, osteopontin, alkaline phosphatase, and type I collagen mRNA in long-term cultures of fetal rat calvarial osteoblasts. J Bone Miner Res 1994;9:855-63.
[18] Midy V, Plouet J. Vasculotropin/vascular endothelial growth factor induces differentiation in cultured osteoblasts. Biochem Biophys Res Commun 1994;199:380-6.
[19] Mundy GR. Nutritional modulators of bone remodeling during aging. Am J Clin Nutr 2006;83:427S-30S.
[20] Rogers MJ. Statins: lower lipids and better bones? Nat Med 2000;6:21-3.
[21] Rogers MJ, Xiong X, Brown RJ, Watts DJ, Russell RG, Bayless AV, et al. Structure-activity relationships of new heterocycle-containing bisphosphonates as inhibitors of bone resorption and as inhibitors of growth of Dictyostelium discoideum amoebae. Mol Pharmacol 1995;47:398-402.
[22] Rogers MJ, Chilton KM, Coxon FP, Lawry J, Smith MO, Suri S, et al. Bisphosphonates induce apoptosis in mouse macrophage-like cells in vitro by a nitric oxide-independent mechanism. J Bone Miner Res 1996;11:1482-91.
[23] Staal A, Frith JC, French MH, Swartz J, Gungor T, Harrity TW, et al. The ability of statins to inhibit bone resorption is directly related to their inhibitory effect on HMG-CoA reductase activity. J Bone Miner Res 2003;18:88-96.
[24] Jadhav SB, Narayana Murthy PS, Singh MM, Jain GK. Distribution of lovastatin to bone and its effect on bone turnover in rats. J Pharm Pharmacol 2006;58:1451-8.
[25] Woo JT, Kasai S, Stern PH, Nagai K. Compactin suppresses bone resorption by inhibiting the fusion of prefusion osteoclasts and disrupting the actin ring in osteoclasts. J Bone Miner Res 2000;15:650-62.
[26] Ahn KS, Sethi G, Chaturvedi MM, Aggarwal BB. Simvastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, suppresses osteoclastogenesis induced by receptor activator of nuclear factor-kappaB ligand through modulation of NF-kappaB pathway. Int J Cancer 2008;123:1733-40.
[27] Yamashita M, Otsuka F, Mukai T, Yamanaka R, Otani H, Matsumoto Y, et al. Simvastatin inhibits osteoclast differentiation induced by bone morphogenetic protein-2 and RANKL through regulating MAPK, AKT and Src signaling. Regul Pept 2010;162:99–108.
[28] Woo JT, Nakagawa H, Krecic AM, Nagai K, Hamilton AD, Sebti SM, et al. Inhibitory effects of mevastatin and a geranylgeranyl transferase I inhibitor (GGTI-2166) on mononuclear osteoclast formation induced by receptor activator of NFκB ligand (RANKL) or tumor necrosis factor-α (TNF-α). Biochem Pharmacol 2005;69:87-95.
[29] Song C, Guo Z, Ma Q, Chen Z, Liu Z, Jia H, et al. Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. Biochem Biophys Res Commun 2003;308:458-62.
[30] Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. Biochem Biophys Res Commun 2001;280:874-7.
[31] Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, et al. Stimulation of bone formation in vitro and in rodents by statins. Science 1999;286:1946-9.
[32] Cho YE, Alcantara E, Kumaran S, Son KH, Sohn HY, Lee JH, et al. Red yeast rice stimulates osteoblast proliferation and increases alkaline phosphatase activity in MC3T3-E1 cells. Nutr Res 2010;30:501-10.
[33] Maeda T, Matsunuma A, Kurahashi I, Yanagawa T, Yoshida H, Horiuchi N. Induction of osteoblast differentiation indices by statins in MC3T3-E1 cells. J Cell Biochem 2004;92:458-71.
[34] Weivoda MM, Hohl RJ. Effects of farnesyl pyrophosphate accumulation on calvarial osteoblast differentiation. Endocrinology 2011;152:3113-22.
[35] Kim IS, Jeong BC, Kim OS, Kim YJ, Lee SE, Lee KN, et al. Lactone form 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) stimulate the osteoblastic differentiation of mouse periodontal ligament cells via the ERK pathway. J Periodontal Res 2011;46:204-13.
[36] Ha H, Lee JH, Kim HN, Lee ZH. alpha-Tocotrienol inhibits osteoclastic bone resorption by suppressing RANKL expression and signaling and bone resorbing activity. Biochem Biophys Res Commun 2011;406:546-51.
[37] Coxon FP, Helfrich MH, Van't Hof R, Sebti S, Ralston SH, Hamilton A, et al. Protein geranylgeranylation is required for osteoclast formation, function, and survival: inhibition by bisphosphonates and GGTI-298. J Bone Miner Res 2000;15:1467-76.
[38] Hiruma Y, Nakahama K, Fujita H, Morita I. Vitamin K(2) and geranylgeraniol, its side chain component, inhibited osteoclast formation in a different manner. Biochem Biophys Res Commun 2004;314:24-30.
[39] Dolder S, Hofstetter W, Wetterwald A, Muhlbauer RC, Felix R. Effect of monoterpenes on the formation and activation of osteoclasts in vitro. J Bone Miner Res 2006;21:647-55.
[40] Sung B, Murakami A, Oyajobi BO, Aggarwal BB. Zerumbone abolishes RANKL-induced NF-kappaB activation, inhibits osteoclastogenesis, and suppresses human breast cancer-induced bone loss in athymic nude mice. Cancer Res 2009;69:1477-84.
[41] Tsubaki M, Satou T, Itoh T, Imano M, Yanae M, Kato C, et al. Bisphosphonate- and statin-induced enhancement of OPG expression and inhibition of CD9, M-CSF, and RANKL expressions via inhibition of the Ras/MEK/ERK pathway and activation of p38MAPK in mouse bone marrow stromal cell line ST2. Mol Cell Endocrinol 2012 In press.
[42] Luckman SP, Coxon FP, Ebetino FH, Russell RG, Rogers MJ. Heterocycle-containing bisphosphonates cause apoptosis and inhibit bone resorption by preventing protein prenylation: evidence from structure-activity relationships in J774 macrophages. J Bone Miner Res 1998;13:1668-78.
[43] Reszka AA, Halasy-Nagy JM, Masarachia PJ, Rodan GA. Bisphosphonates act directly on the osteoclast to induce caspase cleavage of mst1 kinase during apoptosis. A link between inhibition of the mevalonate pathway and regulation of an apoptosis-promoting kinase. J Biol Chem 1999;274:34967-73.
[44] Lazzerini PE, Lorenzini S, Selvi E, Capecchi PL, Chindamo D, Bisogno S, et al. Simvastatin inhibits cytokine production and nuclear factor-κB activation in interleukin 1β-stimulated synoviocytes from rheumatoid arthritis patients. Clin Exp Rheumatol 2007;25:696-700.
[45] Wu SJ, Liu PL, Ng LT. Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. Mol Nutr Food Res 2008;52:921-9.
[46] Wang Y, Jiang Q. γ-Tocotrienol inhibits IL-6 by suppression of C/EBPβ expression and NF-κB signaling in lipopolysaccharide-stimulated macrophages. FASEB J 2011;25:lb178.
[47] Abeles AM, Marjanovic N, Park J, Attur M, Chan ES, Al-Mussawir HE, et al. Protein isoprenylation regulates secretion of matrix metalloproteinase 1 from rheumatoid synovial fibroblasts: effects of statins and farnesyl and geranylgeranyl transferase inhibitors. Arthritis Rheum 2007;56:2840-53.
[48] Yam ML, Abdul Hafid SR, Cheng HM, Nesaretnam K. Tocotrienols suppress proinflammatory markers and cyclooxygenase-2 expression in RAW264.7 macrophages. Lipids 2009;44:787-97.
[49] Duque G, Vidal C, Rivas D. Protein isoprenylation regulates osteogenic differentiation of mesenchymal stem cells: effect of alendronate, and farnesyl and geranylgeranyl transferase inhibitors. Br J Pharmacol 2011;162:1109-18.
[50] Li X, Cui Q, Kao C, Wang GJ, Balian G. Lovastatin inhibits adipogenic and stimulates osteogenic differentiation by suppressing PPARgamma2 and increasing Cbfa1/Runx2 expression in bone marrow mesenchymal cell cultures. Bone 2003;33:652-9.
[51] Ohnaka K, Shimoda S, Nawata H, Shimokawa H, Kaibuchi K, Iwamoto Y, et al. Pitavastatin enhanced BMP-2 and osteocalcin expression by inhibition of Rho-associated kinase in human osteoblasts. Biochem Biophys Res Commun 2001;287:337-42.
[52] Rosen CJ, Bilezikian JP. Clinical review 123: Anabolic therapy for osteoporosis. J Clin Endocrinol Metab 2001;86:957-64.
[53] Bauer DC. HMG CoA reductase inhibitors and the skeleton: a comprehensive review. Osteoporos Int 2003;14:273-82.
[54] Leem K, Park SY, Lee DH, Kim H. Lovastatin increases longitudinal bone growth and bone morphogenetic protein-2 levels in the growth plate of Sprague–Dawley rats. Eur J Pediatr 2002;161:406-7.
[55] Garrett IR, Mundy GR. The role of statins as potential targets for bone formation. Arthritis Res 2002;4:237-40.
[56] Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S. Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. Biochem Biophys Res Commun 2000;271:688-92.
[57] Maeda T, Kawane T, Horiuchi N. Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. Endocrinology 2003;144:681-92.
[58] Ruiz-Gaspa S, Nogues X, Enjuanes A, Monllau JC, Blanch J, Carreras R, et al. Simvastatin and atorvastatin enhance gene expression of collagen type 1 and osteocalcin in primary human osteoblasts and MG-63 cultures. J Cell Biochem 2007;101:1430-8.
[59] Liu S, Bertl K, Sun H, Liu ZH, Andrukhov O, Rausch-Fan X. Effect of simvastatin on the osteogenetic behavior of alveolar osteoblasts and periodontal ligament cells. Hum Cell 2012;25:29-35.
[60] Wong RW, Rabie B. Chinese red yeast rice (*Monascus purpureus*-fermented rice) promotes bone formation. Chin Med 2008;3:4.
[61] Fernandes NV, Guntipalli PK, Mo H. *d*-δ-Tocotrienol-mediated cell cycle arrest and apoptosis in human melanoma cells. Anticancer Res 2010;30:4937-44.
[62] Yano Y, Satoh H, Fukumoto K, Kumadaki I, Ichikawa T, Yamada K, et al. Induction of cytotoxicity in human lung adenocarcinoma cells by 6-O-carboxypropyl-α-tocotrienol, a redox-silent derivative of α-tocotrienol. Int J Cancer 2005;115:839-46.

[63] Parker RA, Pearce BC, Clark RW, Gordon DA, Wright JJ. Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 1993;268: 11230-8.
[64] Song BL, DeBose-Boyd RA. Insig-dependent ubiquitination and degradation of 3-hydroxy-3-methylglutaryl coenzyme a reductase stimulated by δ- and γ-tocotrienols. J Biol Chem 2006;281:25054-61.
[65] Qureshi AA, Burger WC, Peterson DM, Elson CE. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. J Biol Chem 1986;261:10544-50.
[66] Pearce BC, Parker RA, Deason ME, Qureshi AA, Wright JJ. Hypocholesterolemic activity of synthetic and natural tocotrienols. J Med Chem 1992;35:3595-606.
[67] Fisher JE, Rodan GA, Reszka AA. In vivo effects of bisphosphonates on the osteoclast mevalonate pathway. Endocrinology 2000;141:4793-6.
[68] Correll CC, Edwards PA. Mevalonic acid-dependent degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase in vivo and in vitro. J Biol Chem 1994;269: 633-8.
[69] Correll CC, Ng L, Edwards PA. Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J Biol Chem 1994;269: 17390-3.
[70] Sever N, Song BL, Yabe D, Goldstein JL, Brown MS, DeBose-Boyd RA. Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methyl-glutaryl-CoA reductase stimulated by sterols and geranylgeraniol. J Biol Chem 2003;278:52479-90.
[71] Houten SM, Schneiders MS, Wanders RJ, Waterham HR. Regulation of isoprenoid/cholesterol biosynthesis in cells from mevalonate kinase-deficient patients. J Biol Chem 2003;278:5736-43.
[72] Miquel K, Pradines A, Favre G. Farnesol and geranylgeraniol induce actin cytoskeleton disorganization and apoptosis in A549 lung adenocarcinoma cells. Biochem Biophys Res Commun 1996;225:869-76.
[73] Katuru R, Fernandes NV, Elfakhani M, Dutta D, Mills N, Hynds DL, et al. Mevalonate depletion mediates the suppressive impact of geranylgeraniol on murine B16 melanoma cells. Exp Biol Med (Maywood) 2011;236:604-13.
[74] Clegg RJ, Middleton B, Bell GD, White DA. Inhibition of hepatic cholesterol synthesis and S-3-hydroxy-3-methylglutaryl-CoA reductase by mono and bicyclic monoterpenes administered in vivo. Biochem Pharmacol 1980;29: 2125-7.
[75] Clegg RJ, Middleton B, Bell GD, White DA. The mechanism of cyclic monoterpene inhibition of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase in vivo in the rat. J Biol Chem 1982;257:2294-9.
[76] Peffley DM, Gayen AK. Plant-derived monoterpenes suppress hamster kidney cell 3-hydroxy-3-methylglutaryl coenzyme a reductase synthesis at the post-transcriptional level. J Nutr 2003;133:38-44.
[77] Keller RK, Fliesler SJ. Mechanism of aminobisphosphonate action: characterization of alendronate inhibition of the isoprenoid pathway. Biochem Biophys Res Commun 1999;266:560-3.
[78] Grasser WA, Baumann AP, Petras SF, Harwood Jr HJ, Devalaraja R, Renkiewicz R, et al. Regulation of osteoclast differentiation by statins. J Musculoskelet Neuronal Interact 2003;3:53-62.
[79] Westfall D, Aboushadi N, Shackelford JE, Krisans SK. Metabolism of farnesol: phosphorylation of farnesol by rat liver microsomal and peroxisomal fractions. Biochem Biophys Res Commun 1997;230:562-8.
[80] Bentinger M, Grunler J, Peterson E, Swiezewska E, Dallner G. Phosphorylation of farnesol in rat liver microsomes: properties of farnesol kinase and farnesyl phosphate kinase. Arch Biochem Biophys 1998;353:191-8.
[81] Krause C, de Gorter DJJ, Karperien M, ten Dijke P. Signal transduction cascades controlling osteoblast differentiation. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism: American Society for Bone and Mineral Research; 2008. p. 10–6.
[82] Ahmad NS, Khalid BA, Luke DA, Ima Nirwana S. Tocotrienol offers better protection than tocopherol from free radical-induced damage of rat bone. Clin Exp Pharmacol Physiol 2005;32:761-70.
[83] Hermizi H, Faizah O, Ima-Nirwana S, Ahmad Nazrun S, Norazlina M. Beneficial effects of tocotrienol and tocopherol on bone histomorphometric parameters in Sprague–Dawley male rats after nicotine cessation. Calcif Tissue Int 2009;84: 65-74.
[84] Akiyama Y, Hara K, Kobayashi M, Tomiuga T, Nakamura T. Inhibitory effect of vitamin K2 (menatetrenone) on bone resorption in ovariectomized rats: a histomorphometric and dual energy X-ray absorptiometric study. Jpn J Pharmacol 1999;80:67-74.
[85] Muhlbauer RC, Lozano A, Palacio S, Reinli A, Felix R. Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone 2003;32: 372-80.
[86] Ayukawa Y, Yasukawa E, Moriyama Y, Ogino Y, Wada H, Atsuta I, et al. Local application of statin promotes bone repair through the suppression of osteoclasts and the enhancement of osteoblasts at bone-healing sites in rats. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:336-42.
[87] Ayukawa Y, Ogino Y, Moriyama Y, Atsuta I, Jinno Y, Kihara M, et al. Simvastatin enhances bone formation around titanium implants in rat tibiae. J Oral Rehabil 2010;37:123-30.
[88] Shuid AN, Mehat Z, Mohamed N, Muhammad N, Soelaiman IN. Vitamin E exhibits bone anabolic actions in normal male rats. J Bone Miner Metab 2010;28:149-56.
[89] Gutierrez GE, Edwards JR, Garrett IR, Nyman JS, McCluskey B, Rossini G, et al. Transdermal lovastatin enhances fracture repair in rats. J Bone Miner Res 2008;23:1722-30.
[90] Wang GJ, Chung KC, Shen WJ. Lipid clearing agents in steroid-induced osteoporosis. J Formos Med Assoc 1995;94:589-92.
[91] Norazlina M, Ng FW, Ima-Nirwana S. γ-tocotrienol is required for normal vitamin D metabolism in female rats. Indian J Pharmacol 2005;37:309-14.
[92] Norazlina M, Ima-Nirwana S, Abul Gapor MT, Abdul Kadir Khalid B. Tocotrienols are needed for normal bone calcification in growing female rats. Asia Pac J Clin Nutr 2002;11:194-9.
[93] Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. JAMA 2000;283:3205-10.
[94] Chan KA, Andrade SE, Boles M, Buist DS, Chase GA, Donahue JG, et al. Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. Lancet 2000;355:2185-8.
[95] Wang PS, Solomon DH, Mogun H, Avorn J. HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. JAMA 2000;283:3211-6.
[96] Rejnmark L, Olsen ML, Johnsen SP, Vestergaard P, Sorensen HT, Mosekilde L. Hip fracture risk in statin users — a population-based Danish case–control study. Osteoporos Int 2004;15:452-8.
[97] Schoofs MW, Sturkenboom MC, van der Klift M, Hofman A, Pols HA, Stricker BH. HMG-CoA reductase inhibitors and the risk of vertebral fracture. J Bone Miner Res 2004;19:1525-30.
[98] Nakashima A, Nakashima R, Ito T, Masaki T, Yorioka N. HMG-CoA reductase inhibitors prevent bone loss in patients with Type 2 diabetes mellitus. Diabet Med 2004;21:1020-4.
[99] Edwards CJ, Hart DJ, Spector TD. Oral statins and increased bone-mineral density in postmenopausal women. Lancet 2000;355:2218-9.
[100] Tang QO, Tran GT, Gamie Z, Graham S, Tsialogiannis E, Tsiridis E, et al. Statins: under investigation for increasing bone mineral density and augmenting fracture healing. Expert Opin Investig Drugs 2008;17:1435-63.
[101] Kuzuya M, Suzuki Y, Asai T, Koike T, Kanda S, Nakamura A, et al. Atorvastatin, 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, reduces bone resorption in the elderly. J Am Geriatr Soc 2003;51:1677-8.
[102] Funkhouser HL, Adera T, Adler RA. Effect of HMG-CoA reductase inhibitors (statins) on bone mineral density. J Clin Densitom 2002;5:151-8.
[103] Hatzigeorgiou C, Jackson JL. Hydroxymethylglutaryl-coenzyme A reductase inhibitors and osteoporosis: a meta-analysis. Osteoporos Int 2005;16:990-8.
[104] Chung YS, Lee MD, Lee SK, Kim HM, Fitzpatrick LA. HMG-CoA reductase inhibitors increase BMD in type 2 diabetes mellitus patients. J Clin Endocrinol Metab 2000;85:1137-42.
[105] LaCroix AZ, Cauley JA, Pettinger M, Hsia J, Bauer DC, McGowan J, et al. Statin use, clinical fracture, and bone density in postmenopausal women: results from the Women's Health Initiative Observational Study. Ann Intern Med 2003;139: 97–104.
[106] van Staa TP, Wegman S, de Vries F, Leufkens B, Cooper C. Use of statins and risk of fractures. JAMA 2001;285:1850-5.
[107] Pedersen TR, Kjekshus J. Statin drugs and the risk of fracture. 4S Study Group. JAMA 2000;284:1921-2.
[108] Lupattelli G, Scarponi AM, Vaudo G, Siepi D, Roscini AR, Gemelli F, et al. Simvastatin increases bone mineral density in hypercholesterolemic postmenopausal women. Metabolism 2004;53:744-8.
[109] Montagnani A, Gonnelli S, Cepollaro C, Pacini S, Campagna MS, Franci MB, et al. Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholesterolemic postmenopausal women: a 1-year longitudinal study. Bone 2003;32:427-33.
[110] Bauer DC, Mundy GR, Jamal SA, Black DM, Cauley JA, Ensrud KE, et al. Use of statins and fracture: results of 4 prospective studies and cumulative meta-analysis of observational studies and controlled trials. Arch Intern Med 2004;164:146-52.
[111] Chan MH, Mak TW, Chiu RW, Chow CC, Chan IH, Lam CW. Simvastatin increases serum osteocalcin concentration in patients treated for hypercholesterolaemia. J Clin Endocrinol Metab 2001;86:4556-9.
[112] Sirola J, Honkanen R, Kroger H, Jurvelin JS, Maenpaa P, Saarikoski S. Relation of statin use and bone loss: a prospective population-based cohort study in early postmenopausal women. Osteoporos Int 2002;13:537-41.
[113] Pasco JA, Kotowicz MA, Henry MJ, Sanders KM, Nicholson GC. Statin use, bone mineral density, and fracture risk: Geelong Osteoporosis Study. Arch Intern Med 2002;162:537-40.
[114] Orimo H, Shiraki M, Tomita A, Morii H, Fujita T, Ohata M. Effects of menatetrenone on the bone and calcium metabolism in osteoporosis: a double-blind placebo-controlled study. J Bone Miner Metab 1998;16:106-12.
[115] Watanabe S, Fukumoto S, Takeuchi Y, Fujita H, Nakano T, Fujita T. Effects of 1-year treatment with fluvastatin or pravastatin on bone. Am J Med 2001;110: 584-7.
[116] Berthold HK, Unverdorben S, Zittermann A, Degenhardt R, Baumeister B, Unverdorben M, et al. Age-dependent effects of atorvastatin on biochemical bone turnover markers: a randomized controlled trial in postmenopausal women. Osteoporos Int 2004;15:459-67.
[117] Jadhav SB, Jain GK. Statins and osteoporosis: new role for old drugs. J Pharm Pharmacol 2006;58:3–18.
[118] Reid IR, Hague W, Emberson J, Baker J, Tonkin A, Hunt D, et al. Effect of pravastatin on frequency of fracture in the LIPID study: secondary analysis of a randomised controlled trial. Long-term Intervention with Pravastatin in Ischaemic Disease. Lancet 2001;357:509-12.
[119] Pedersen TR, Berg K, Cook TJ, Faergeman O, Haghfelt T, Kjekshus J, et al. Safety and tolerability of cholesterol lowering with simvastatin during 5 years in the Scandinavian Simvastatin Survival Study. Arch Intern Med 1996;156:2085-92.

[120] Rejnmark L, Buus NH, Vestergaard P, Heickendorff L, Andreasen F, Larsen ML, et al. Effects of simvastatin on bone turnover and BMD: a 1-year randomized controlled trial in postmenopausal osteopenic women. J Bone Miner Res 2004;19:737-44.
[121] Cummings SR, Bauer DC. Do statins prevent both cardiovascular disease and fracture? JAMA 2000;283:3255-7.
[122] Roelofs AJ, Thompson K, Gordon S, Rogers MJ. Molecular mechanisms of action of bisphosphonates: current status. Clin Cancer Res 2006;12:6222s-30s.
[123] Bergstrom JD, Bostedor RG, Masarachia PJ, Reszka AA, Rodan G. Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. Arch Biochem Biophys 2000;373:231-41.
[124] Rogers MJ, Frith JC, Luckman SP, Coxon FP, Benford HL, Monkkonen J, et al. Molecular mechanisms of action of bisphosphonates. Bone 1999;24:73S-9S.
[125] Benford HL, Frith JC, Auriola S, Monkkonen J, Rogers MJ. Farnesol and geranylgeraniol prevent activation of caspases by aminobisphosphonates: biochemical evidence for two distinct pharmacological classes of bisphosphonate drugs. Mol Pharmacol 1999;56:131-40.
[126] Coxon FP, Thompson K, Rogers MJ. Recent advances in understanding the mechanism of action of bisphosphonates. Curr Opin Pharmacol 2006;6: 307-12.
[127] Akiyama Y, Hara K, Tajima T, Murota S, Morita I. Effect of vitamin K2 (menatetrenone) on osteoclast-like cell formation in mouse bone marrow cultures. Eur J Pharmacol 1994;263:181-5.
[128] Hara K, Akiyama Y, Nakamura T, Murota S, Morita I. The inhibitory effect of vitamin K2 (menatetrenone) on bone resorption may be related to its side chain. Bone 1995;16:179-84.
[129] Taira H, Fujikawa Y, Kudo O, Itonaga I, Torisu T. Menatetrenone (vitamin K_2) acts directly on circulating human osteoclast precursors. Calcif Tissue Int 2003;73: 78-85.
[130] Bach TJ. Some new aspects of isoprenoid biosynthesis in plants–a review. Lipids 1995;30:191-202.
[131] Elson CE, Peffley DM, Hentosh P, Mo H. Isoprenoid-mediated inhibition of mevalonate synthesis: potential application to cancer. Proc Soc Exp Biol Med 1999;221:294-311.
[132] Mo H, Elson CE. Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention. Exp Biol Med (Maywood) 2004;229:567-85.
[133] Crowell PL, Elson CE. Isoprenoids, health and disease. In: Wildman REC, editor. Handbook of nutraceuticals and functional foods CRC series in modern nutrition. Boca Raton: CRC Press; 2001. p. 31-53.
[134] Mo H, Elson CE. Isoprenoids and novel inhibitors of mevalonate pathway activities. In: Heber D, Blackburn GL, Go VLW, Milner J, editors. Nutritional oncology. Burlington: Academic Press; 2006. p. 629-44.
[135] Qureshi AA, Mangels AR, Din ZZ, Elson CE. Inhibition of hepatic mevalonate biosynthesis by the monoterpene, *d*-limonene. J Agric Food Chem 1988;36: 1220-4.
[136] Tatman D, Mo H. Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. Cancer Lett 2002;175:129-39.
[137] Gao YH, Yamaguchi M. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow cultures. Biochem Pharmacol 1999;58: 767-72.
[138] Karieb S, Fox SW. Phytoestrogens directly inhibit TNF-alpha-induced bone resorption in RAW264.7 cells by suppressing c-fos-induced NFATc1 expression. J Cell Biochem 2011;112:476-87.
[139] Sliwinski L, Folwarczna J, Janiec W, Grynkiewicz G, Kuzyk K. Differential effects of genistein, estradiol and raloxifene on rat osteoclasts in vitro. Pharmacol Rep 2005;57:352-9.
[140] Sung JH, Choi SJ, Lee SW, Park KH, Moon TW. Isoflavones found in Korean soybean paste as 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitors. Biosci Biotechnol Biochem 2004;68:1051-8.
[141] Sung JH, Lee SJ, Park KH, Moon TW. Isoflavones inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase in vitro. Biosci Biotechnol Biochem 2004;68:428-32.
[142] Lanham-New SA. Fruit and vegetables: the unexpected natural answer to the question of osteoporosis prevention? Am J Clin Nutr 2006;83:1254-5.
[143] Muhlbauer RC, Li F. Effect of vegetables on bone metabolism. Nature 1999;401: 343-4.
[144] Prynne CJ, Mishra GD, O'Connell MA, Muniz G, Laskey MA, Yan L, et al. Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. Am J Clin Nutr 2006;83:1420-8.
[145] Anderson JJ. Plant-based diets and bone health: nutritional implications. Am J Clin Nutr 1999;70:539S-42S.
[146] New SA, Robins SP, Campbell MK, Martin JC, Garton MJ, Bolton-Smith C, et al. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable consumption and bone health? Am J Clin Nutr 2000;71:142-51.
[147] Hussein D, Mo H. *d*-δ-Tocotrienol-mediated suppression of the proliferation of human PANC-1, MIA PaCa2 and BxPC-3 pancreatic carcinoma cells. Pancreas 2009;38:e124-36.
[148] Mo H, Elson CE. Role of the mevalonate pathway in tocotrienol-mediated tumor suppression. In: Watson RR, Preedy VR, editors. Tocotrienols: vitamin E beyond tocopherols. Boca Raton: CRC Press; 2008. p. 185-207.
[149] Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. Mol Aspects Med 2007;28:692-728.
[150] Yang B, Ahotupa M, Määttä P, Kallio H. Composition and antioxidative activities of supercritical CO2-extracted oils from seeds and soft parts of northern berries. Food Res Int 2011;44:2009-17.
[151] Helbig D, Böhm V, Wagner A, Schubert R, Jahreis G. Berry seed press residues and their valuable ingredients with special regard to black currant seed press residues. Food Chem 2008;111:1043-9.
[152] Chun J, Lee J, Ye L, Exler J, Eitenmiller RR. Tocopherol and tocotrienol contents of raw and processed fruits and vegetables in the United States diet. J Food Comp Analy 2006;19:196-204.
[153] Choi Y, Lee J. Antioxidant and antiproliferative properties of a tocotrienol-rich fraction from grape seeds. Food Chem 2009;114:1386-90.
[154] Isanga J, Zhang G-N. Biologically active components and nutraceuticals in peanuts and related products: review. Food Rev Int 2007;23:123-40.
[155] Amaral JS, Casal S, Alves MR, Seabra RM, Oliveira BP. Tocopherol and tocotrienol content of hazelnut cultivars grown in Portugal. J Agric Food Chem 2006;54: 1329-36.
[156] Harinantenaina L. Tocotrienols in plants: sources and importance. In: Watson RR, Preedy VR, editors. Tocotrienols: vitamin E beyond tocopherols. Boca Raton: CRC Press; 2008. p. 43-60.
[157] Franke AA, Murphy SP, Lacey R, Custer LJ. Tocopherol and tocotrienol levels of foods consumed in Hawaii. J Agric Food Chem 2007;55:769-78.
[158] Hassapidou MN, Manoukas AG. Tocopherol and tocotrienol compositions of raw table olive fruit. J Sci Food Agric 1993;61:277-80.
[159] Nesaretnam K. Multitargeted therapy of cancer by tocotrienols. Cancer Lett 2008;269:388-95.
[160] Frega N, Mozzon M, Bocci F. Identification and estimation of tocotrienols in the annatto lipid fraction by gas chromatography–mass spectrometry. J Am Oil Chem Soc 1998;75:1723-7.
[161] Burger WC, Qureshi AA, Prentice N, Elson CE. Effects of different fractions of the barley kernel on the hepatic lipid metabolism of chickens. Lipids 1982;17:956-63.
[162] Burger WC, Qureshi AA, Din ZZ, Abuirmeileh N, Elson CE. Suppression of cholesterol biosynthesis by constituents of barley kernel. Atherosclerosis 1984;51:75-87.
[163] Qureshi AA, Pearce BC, Nor RM, Gapor A, Peterson DM, Elson CE. Dietary α-tocopherol attenuates the impact of γ-tocotrienol on hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in chickens. J Nutr 1996;126: 389-94.
[164] Qureshi AA, Peterson DM, Elson CE, Mangels AR, Din ZZ. Stimulation of avian cholesterol metabolism by α-tocopherol. Nutr Rep Int 1989;40:993–1001.
[165] Nizar AM, Nazrun AS, Norazlina M, Norliza M, Ima Nirwana S. Low dose of tocotrienols protects osteoblasts against oxidative stress. Clin Ter 2011;162: 533-8.
[166] Ima-Nirwana S, Suhaniza S. Effects of tocopherols and tocotrienols on body composition and bone calcium content in adrenalectomized rats replaced with dexamethasone. J Med Food 2004;7:45-51.
[167] Mehat MZ, Shuid AN, Mohamed N, Muhammad N, Soelaiman IN. Beneficial effects of vitamin E isomer supplementation on static and dynamic bone histomorphometry parameters in normal male rats. J Bone Miner Metab 2010;28:503-9.
[168] Ima-Nirwana S, Nurshazwani Y, Nazrun AS, Norliza M, Norazlina M. Subacute and subchronic toxicity studies of palm vitamin E in mice. J Pharmacol Toxicol 2011;6:166-73.
[169] Deyhim F, Garcia C, Villarreal A, Garcia K, Rios R, Gonzales C, et al. Vitamin E does not support bone quality in orchidectomized rats. Curr Nutr Food Sci 2007;3: 300-3.
[170] Norazlina M, Lee PL, Lukman HI, Nazrun AS, Ima-Nirwana S. Effects of vitamin E supplementation on bone metabolism in nicotine-treated rats. Singapore Med J 2007;48:195-9.
[171] Shuid AN, Chuan LH, Mohamed N, Jaarin K, Fong YS, Soelaiman IN. Recycled palm oil is better than soy oil in maintaining bone properties in a menopausal syndrome model of ovariectomized rat. Asia Pac J Clin Nutr 2007;16:393-402.
[172] Fujita K, Iwasaki M, Ochi H, Fukuda T, Ma C, Miyamoto T, et al. Vitamin E decreases bone mass by stimulating osteoclast fusion. Nat Med 2012;18:589-94.
[173] Berchtold CM, Chen KS, Miyamoto S, Gould MN. Perillyl alcohol inhibits a calcium-dependent constitutive nuclear factor-kappaB pathway. Cancer Res 2005;65:8558-66.
[174] Ji X, Gupta S. Delta-Tocotrienol enhances cisplatin-induced inhibition of growth and invasion of Non-Small Cell Lung Cancer Cells. FASEB J 2012;26: 822.23.
[175] Husain K, Centeno B, Perez M, Lee G, Kazim S, Chen D, et al. Delta-tocotrienol delays the progression of pancreatic intraepithelial neoplasia (PanIN) lesions in a conditional KrasG12D mouse model. Am Assoc Cancer Res 2012;53 Abstract# 1613.
[176] Ahn KS, Sethi G, Krishnan K, Aggarwal BB. γ-Tocotrienol inhibits nuclear factor-κB signaling pathway through inhibition of receptor-interacting protein and TAK1 leading to suppression of antiapoptotic gene products and potentiation of apoptosis. J Biol Chem 2007;282:809-20.
[177] Wali VB, Sylvester PW. Synergistic antiproliferative effects of γ-tocotrienol and statin treatment on mammary tumor cells. Lipids 2007;42:1113-23.
[178] McAnally JA, Gupta J, Sodhani S, Bravo L, Mo H. Tocotrienols potentiate lovastatin-mediated growth suppression in vitro and in vivo. Exp Biol Med (Maywood) 2007;232:523-31.
[179] Mo H, Elson CE. Apoptosis and cell-cycle arrest in human and murine tumor cells are initiated by isoprenoids. J Nutr 1999;129:804-13.
[180] He L, Mo H, Hadisusilo S, Qureshi AA, Elson CE. Isoprenoids suppress the growth of murine B16 melanomas in vitro and in vivo. J Nutr 1997;127:668-74.

[181] Mo H, Peffley DM, Elson CE. Targeting the action of isoprenoids and related phytochemicals to tumors. In: Heber D, Blackburn GL, Go VLW, editors. Nutritional Oncology. San Diego: Academic Press; 1999. p. 379-91.
[182] Cao J, Qi W, Song BL. Tocotrienols and the regulation of cholesterol biosynthesis. In: Watson RR, Preedy VR, editors. Tocotrienols: vitamin E beyond tocopherols. Boca Raton: CRC Press; 2008. p. 237-56.
[183] Das S, Das M, Das DK. Vitamin E isomers, tocotrienols, in cardioprotection. In: Watson RR, Preedy VR, editors. Tocotrienols: vitamin E beyond tocopherols. Boca Raton: CRC Press; 2008. p. 285-93.
[184] Sen CK, Khanna S, Roy S. Tocotrienols as natural neuroprotective vitamins. In: Watson RR, Preedy VR, editors. Tocotrienols: vitamin E beyond tocopherols. Boca Raton: CRC Press; 2008. p. 361-77.