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# Vitamin Regulation of Osteoclast Differentiation

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**Objectives:** Vitamins play critical roles in cellular metabolism, growth, and many enzymatic processes of the human body. They are also crucial in signal transduction and transcription pathways of many processes, including osteoclast differentiation. This review focused on the positive or negative effect of vitamins on osteoclast differentiation in vivo and in vitro, especially signal transduction. **Methods:** A systematic review of the literature regarding the contributions of the osteoclast differentiation and vitamins was performed, and the most relevant findings on the effect of vitamins on osteoclast differentiation were selected. **Results:** Vitamin D, E, B1, B5, B6, and B12 have mainly anti-osteoporotic effects; however, their mechanism on osteoclast differentiation and activation are variable. Vitamins A and C have been considered to activate osteoclast differentiation and function, but some report a suppressive effect on osteoclast function. Vitamin K and B2 exert an inhibitory effect on osteoclast differentiation and activation both in vitro and in vivo. In contrast, a direct action of niacin, biotin, and folic acid on osteoclast differentiation and activation remains unclear. **Conclusions:** Collectively, vitamins act on osteoclast differentiation and function in various ways depending on cell type, cell maturation and microenvironment.

**Keywords:** Osteoclast, Cell signaling, Vitamin, NFATc1

## Introduction

Bone is a specialized connective tissue that is dynamic and is continuously being remodeled. It is composed of cells and mineralized bone matrix. The bone matrix consists of collagen fibers, ground substance, and inorganic crystalline minerals and salts, with three principal cells in bone: osteoblasts, osteocytes, and osteoclasts [1]. Bone provides important functions in the body as locomotion, structural support and protection of inner organs, retains bone marrow and is a dynamic reservoir of calcium and phosphate. Although seemingly inert, bone is a highly active tissue that is continuously degraded by osteoclasts and neoformed by osteoblasts [2].

Osteoclasts, discovered by Kolliker in 1873 [3], are specialized bone cells of myeloid origin that participate in skeletal turnover by resorbing bone and act in concert with osteoblasts and osteocytes, the other cells that make up bone tissue. Osteoclasts are critical to bone remodeling and repair, including micro-damage, daily wear, and tear, even play an important role in fracture healing [4]. Mature osteoclasts secrete proteases and acids that mediate their resorptive activity [5]. Osteoclasts are terminally differentiated multinucleated cells, which originate tissue macrophage cells derived from hematopoietic progenitors [6, 7]. In their initiation, mononuclear macrophages differentiate to preosteoclasts and then fuse, becoming multinucleated cells [8]. This osteoclastogenic differentiation depends on two principal factors: the macrophage colony-stimulating factor (M-CSF), secreted by osteoblasts and mesenchymal cells [9, 10], and the receptor activator NF-κB (RANK) ligand (RANKL), secreted by osteoblasts, osteocytes, and stromal cells [11, 12]. Together, these factors promote the activation of transcription factors and gene expression in osteoclastogenic differentiation [1, 13].

Osteoclast function consists of several processes: recognition of mineralized tissues, polarization (development of ruffled borders and sealing zones), secretion of acids and proteolytic enzymes into the space beneath the ruffled border, and incorporation and secretion of bone degradation products using the transcytosis system [14, 15]. The expression of several other genes exerts a significant influence on osteoclast differentiation and bone-resorbing activation [13, 16]. The osteoclast is vital not only for bone remodeling but is also essential for bone development. Chondroclasts are one type of osteoclast essential for bone ossification during bone development [17, 18]. During endochondral ossification, cartilage (an avascular tissue) is gradually converted into bone, one of the most highly vascularized tissues in the vertebrate body. The extracellular matrix produced by the terminally differentiated hypertrophic chondrocytes is calcified. This mineralized matrix is partially degraded by chondroclasts and preosteoclasts. Following matrix degradation and permissive blood-vessel invasion, osteogenic progenitors are recruited to the area of cartilage, where they consequently deposit trabecular bone [19, 20].

The abnormal increase in osteoclast formation and activity leads to some bone diseases such as osteoporosis. In some pathologic conditions, including bone metastases and inflammatory arthritis, abnormal osteoclast activation results in periarticular erosions and painful osteolytic lesions [4, 15, 21]. On the other hand, in osteopetrosis, a rare bone disease, genetic mutations that affect formation and resorption functions in osteoclasts lead to decreased bone resorption, resulting in a disproportionate accumulation of bone mass [22, 23].

The osteoclast differentiation and activation are significant in normal bone development and remodeling and pathogenesis of bone disease [24]. These processes are regulated by numerous factors, including hormones, cytokines, transcription factors and micronutrients [25]. Micronutrients are essential dietary elements or organic compounds that are required in only small quantities for normal physiologic processes to occur. They include vitamins and minerals which are consumed in food or dietary supplements.

Vitamins play critical roles in cellular metabolism, growth, and many enzymatic processes of the human body, and are also important in signal transduction and transcription pathways of many functions, including osteoclast differentiation [26, 27]. Therefore, we aimed to review the regulation of vitamins on osteoclast differentiation. This review focused on the positive or negative regulatory effect of vitamins on osteoclast differentiation in vivo and in vitro, especially in signal transduction.

## Materials and Methods

### Subject

A systematic review of the literature regarding the contributions of vitamins on osteoclast differentiation was performed, and the most relevant findings were selected.

### Procedure

The search strategy included searching the electronic databases of Web of Science by Thomson Reuters, PubMed Central Databases, and Google Scholar.

### Search strategy

Relevant studies were identified using the following strategy: one of the words from list 1 was combined with all terms/abbreviations (one after another) from list 2. List 1: bone turnover markers, osteoclast, osteoclastogenesis. List 2: vitamin A, retinol, vitamin D, cholecalciferol, ergocalciferols, vitamin E, tocopherols, tocotrienols, vitamin K, vitamin C, ascorbic acid, ascorbate, vitamin B, vitamin B1, thiamin, aneurin, vitamin B2, riboflavin, niacin, nicotinic acid, nicotinamide, vitamin B5, pantothenate, pantothenic acid, biotin, vitamin B12, cobalamin, folic acid, folates. Over 3000 publications were identified, and cited here were the most relevant and important 107 publications.

### Ethical statement

This study was approved by the Ethics and Research Committees of the Mongolian National University of Medical Sciences (№20/3-02).

## Results

### Osteoclast differentiation and activation biology

M-CSF binds to its receptor (M-CSFR) expressed on osteoclast precursors, stimulating their proliferation and inhibiting their apoptosis [28]. RANKL/RANK/OPG system is a key mediator of osteoclastogenesis [11, 12, 29]. RANKL, which is expressed by osteoblasts, osteocytes, and stromal cells [30], is an essential factor for osteoclast differentiation and activation. The binding of RANKL and RANK on osteoclast progenitor cells triggers osteoclast formation. On the other hand, osteoprotegerin (OPG), which is produced by a wide range of cells, including osteoblasts, stromal cells, is a decoy receptor [11]. It binds to RANKL, preventing the RANK/RANKL interaction and, consequently, inhibiting the osteoclastogenesis (Figure 1).

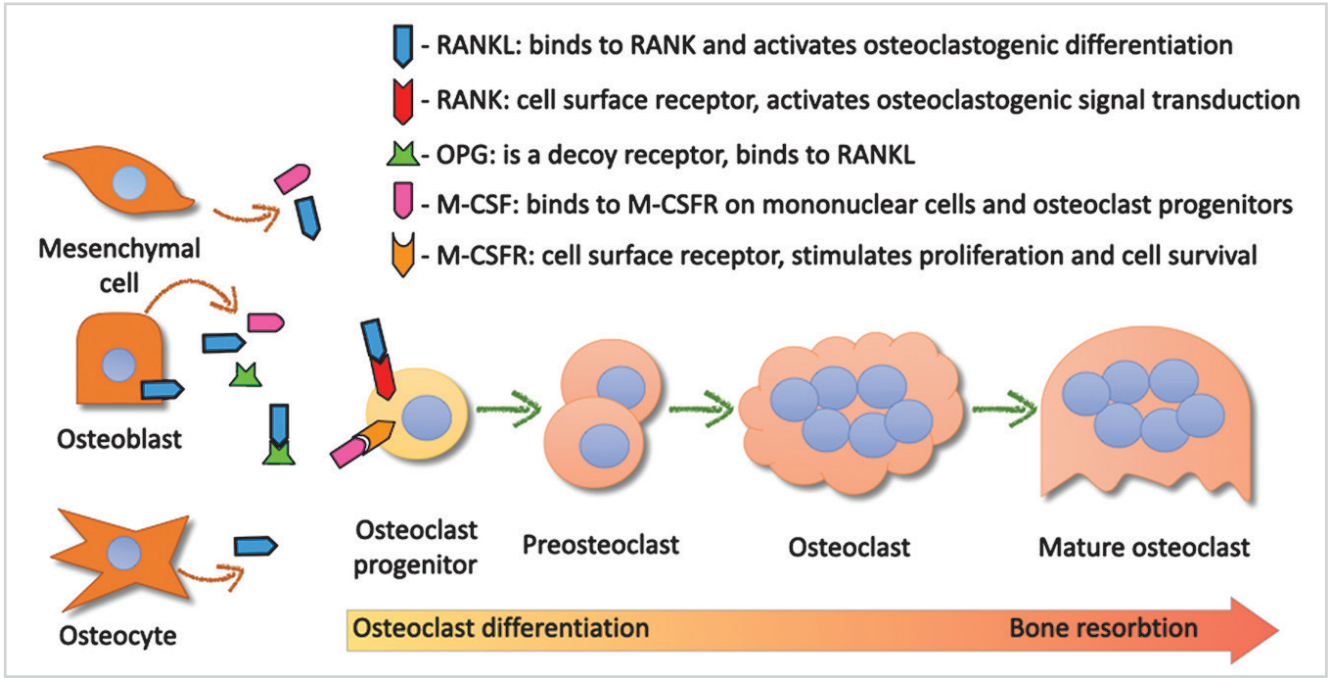


**Figure 1**. HInteraction and development of bone cells for bone resorption. Osteoclast differentiation is induced by RANKL and M-CSF, as well as other cytokines secreted by osteoblasts, osteocytes and mesenchymal cells that control various steps of the osteoclast differentiation, including osteoclast progenitor, preosteoclast (mononuclear), osteoclast (multinuclear) and activated mature osteoclast. OPG secreted by osteoblasts and mesenchymal cell acts as a decoy receptor for RANKL and inhibits osteoclast differentiation.

The RANKL/RANK interaction promotes the activation of tumor necrosis factor receptor-associated factor 6 (TRAF6) and, subsequently, the activation of NF-κB and mitogen-activated protein kinases (MAPKs), such as extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) [31-35]. The nuclear factor of activated T cells (NFATc1) is a downstream transcription factor of the RANKL/RANK signal pathway and plays a crucial role in osteoclastogenesis. By interacting with the transcription factors PU.1, cFos, and microphthalmia-associated transcription factor (MITF), Kruppel-like factor (KLF) 2 and NFATc1 regulate osteoclast-specific genes including TRAP and cathepsin K, which are crucial for osteoclast activity osteoclast-associated receptor upregulation [36-41]. Under the influence of the RANKL/RANK interaction, NFATc1 also induces DC-STAMP expression, which is crucial for the fusion of osteoclast precursors [42, 43] (Figure 2).

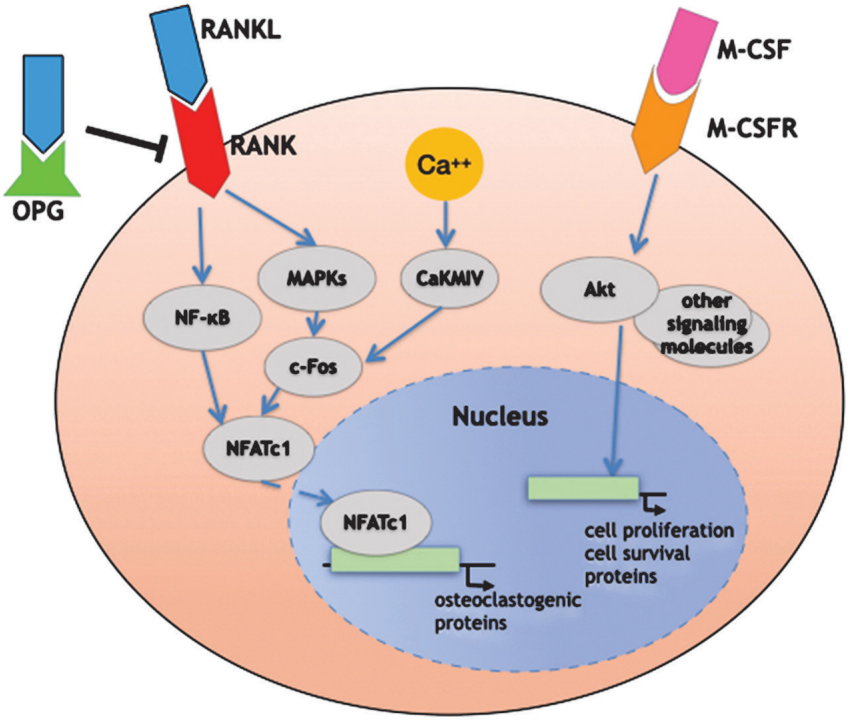


**Figure 2.** Transcriptional regulation of osteoclast differentiation. M-CSF binds to the cell surface receptor M-CSFR and activates downstream signals, critical for the survival, proliferation, and differentiation of early osteoclastic precursor cells. The RANKL and RANK interaction promotes the activation of NF-κB and MAPKs. Activation of c-fos and NF-kB pathways induces the NFATc1 gene. NFATc1 is a downstream transcription factor of the RANKL/RANK signal pathway and plays a crucial role in osteoclastogenesis. The activated NFATc1 translocates and promotes osteoclast-specific genes. This increases the intracellular calcium concentration, which activates the calcium/calmodulin-activated kinases (CaKMIV), leading to the recruitment of NFATc1 to its own promoter which further induces NFATc1.

During bone remodeling, osteoclasts polarize; then, four types of osteoclast membrane domains can be observed at its extracellular interface: the sealing zone and ruffled border that are in contact with the bone matrix, and the basolateral and functional secretory domains, which are not in contact with the bone matrix [14, 44]. Polarization of osteoclasts during bone resorption involves rearrangement of the actin cytoskeleton, in which an F-actin ring that comprises a dense continuous zone of highly dynamic podosome is formed, and consequently, an area of a membrane that develops into the ruffled border is isolated [45, 46]. It is noteworthy that these domains are only formed when osteoclasts are in contact with extracellular mineralized matrix, in a process which αvβ3-integrin (vitronectin receptor, CD51/CD61), as well as the CD44, mediates the attachment of the osteoclast podosomes to the bone surface [47-49]. Ultrastructurally, the ruffled border is a membrane domain formed by microvilli, which is isolated from the surrounded tissue by the clear zone, also known as the sealing zone.

The clear zone is an area devoid of organelles located in the periphery of the osteoclast adjacent to the bone matrix. This sealing zone is formed by an actin ring and several other proteins, including actin, talin, vinculin, paxillin, tensin, and

actin-associated proteins such as α-actinin, fimbrin, gelsolin, and dynamin [2]. The α-integrin binds to noncollagenous bone matrix containing-RGD sequences such as bone sialoprotein, osteopontin, and vitronectin, establishing a peripheric sealing that delimits the central region, where the ruffled border is located [50].

The maintenance of the osteoclast's ruffled border is also essential for bone resorption; this structure is formed by the intense trafficking of lysosomal and endosomal components. In the ruffled border, there is a vacuolar-type H+-ATPase (V-ATPase), which helps to acidify the resorption lacuna (Howship lacuna) and hence to enable dissolution of hydroxyapatite crystals [45, 51, 52]. In this region, protons and enzymes, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K, and matrix metalloproteinase-9 (MMP-9), are transported into the resorption lacuna leading to bone degradation [4, 19, 36]. The products of this degradation are then endocytosed across the ruffled border and transcytosed to the functional secretory domain at the plasma membrane [13] (Figure 3).

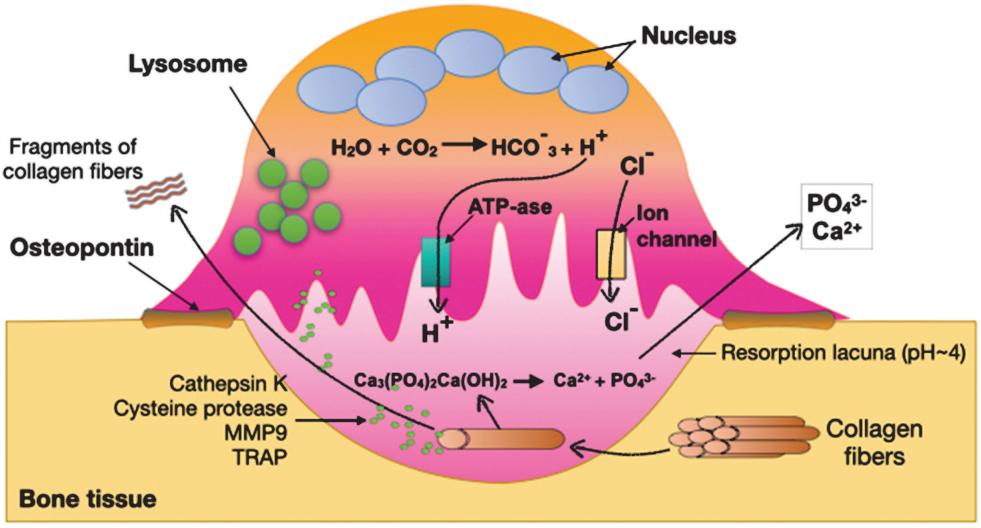


**Figure 3**. Degradation of bone matrix in the resorption lacuna by osteoclast. During bone resorption, H+-protons and chloride ions (Cl–) generated by carbonic anhydrase are transported via the ATPase and ion channel respectively to the ruffled border membrane where they are secreted to acidify the resorption lacuna. Degradation of the demineralized bone matrix occurs through the action of secreted cathepsin K, cysteine protease, matrix metalloproteinases (MMPs), and tartrate-resistant acid phosphatase (TRAP). Bone degradation products are released into the bone microenvironment, internalized into the cell to be degraded by lysosomes, or secreted at the basolateral membrane via transcytosis.

## Endocrine, paracrine regulation of osteoclast differentiation

Osteoclast differentiation and activation are regulated by parathyroid hormone (PTH) from the parathyroid gland, calcitonin from the thyroid gland, and growth factor interleukin 6 (IL-6).

PTH has a direct and indirect effect on osteoclast differentiation and increases osteoclasts in bone fracture callus. PTH promotes both osteoblastic progenitor cells and osteoclastogenesis, thereby enhancing both bone formation and bone remodeling [53].

Calcitonin has a potent inhibitory effect on osteoclast activity via affecting cell attachment and cytoskeletal signal transduction. Calcitonin reduces the contact of osteoclasts with the bone surface and alters osteoclast morphology, while in vitro, calcitonin induces osteoclast retraction and decreases osteoclast mobility [2, 54].

Estrogen upregulates c-Fos and c-Jun within minutes and down-regulates Cathepsin K, lysosomal enzyme secretion (cathepsins and tartrate-resistant alkaline phosphatase), and IL-1R within a few hours [55] Excess glucocorticoids enhance bone resorption by suppressing OPG expression and prolonging the life span of osteoclasts [56].

In the microenvironment, osteoblasts and osteocytes have paracrine interaction with osteoclasts by growth factors, cytokines, and chemokines to maintain the osteoclast differentiation and bone remodeling. These paracrine molecules secreted by osteoblasts and osteocytes divide two groups; one

is pro-osteoclastogenic factors including M-CSF, RANKL [12, 28]; the other is anti-osteoclastogenic factors such as OPG and Semaphorin 3A (SEMA3A), Wnt gene family 5A (WNT5A) and 16 (WNT16)[12, 57-59].

Osteocytes secrete sclerostin (SOST), which inhibits osteoblast differentiation and subsequently, bone formation in a paracrine manner. In the other side, osteoclast secretes regulation molecules that affecting the differentiation and/or functions of osteoblasts and osteocytes, including bone morphogenetic protein 6 (BMP6), collagen triple helix repeat containing 1 (CTHRC1), EphrinB2 (EFNB2), Sphingosine 1-phosphate (S1P), Wnt gene family 10b (WNT10B), Semaphorin 4D (SEMA4D), and Cardiotrophin-1(CT-1) [58-62].

**Fat-soluble vitamins**

There are thirteen known vitamins divided into two classes based on their relative solubility in water and fat. The fat-soluble vitamins A, D, E and K, are absorbed in the intestine in the presence of fat.

**Vitamin A**

Vitamin A, also named retinol, is an essential nutrient needed in small amounts by humans for the normal functioning of the retina, the growth of and differentiation of epithelial tissue, reproduction, the immune response, and bone growth. It is enriched in the liver, egg yolks, and the fat component of dairy products as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids [26]. Vitamin A is converted to an active compound, all-trans-retinoic acid (ATRA), responsible for most of its biological actions. ATRA binds its intracellular nuclear receptors called retinoic acid receptors (RAR).

Retinoids affect both the formation of osteoclasts from precursor cells and the activity of mature osteoclasts. All trans-retinoic acid and its precursors (retinaldehyde) inhibit RANKL-induced osteoclast differentiation in vitro via inhibiting c-Fos, NFATc1, and subsequent RANK expression and antagonizing PPARγ, respectively [63, 64]. In contrast, in organ cultures of bone, including both osteoclast and osteoblast, the dominant effect reported for RAR agonists is the promotion of osteoclast resorptive activity. In bone cultures, ATRA stimulates expression of RANKL, as well as OPG [65, 66]. Vitamin A inhibits RANKL-induced osteoclast differentiation but enhances resorption in co-culture systems. Vitamin A has site-specific effects. It enhances activation of osteoclasts on the periosteal surface of cortical bone, whereas it reduces the osteoclasts formation in the bone marrow in trabecular bone and on endosteal surfaces [67].

**Vitamin D**

Vitamin D includes both cholecalciferol and ergocalciferols and acts on vitamin D receptors to regulate calcium in opposition to PTH. Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are, in turn, needed for the normal mineralization of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body [26]. Vitamin D is converted to the active form 1,25 dihydroxy vitamin D [1,25-(OH)2D], or calcitriol. This active form regulates the transcription of several vitamin D-dependent genes, which code for calcium-transporting proteins and bone matrix proteins. It can also be viewed as a hormone since it can be formed in the skin by the ultraviolet rays' action upon the precursors as 7-dehydrocholesterol and ergosterol [26, 68].

Vitamin D-deficient rats with osteomalacia have reduced the number and activity of osteoclast [14]. 1,25(OH)2D3 at nM concentrations inhibited osteoclast formation in bone marrow macrophage cultures treated with RANKL and M-CSF. However, presently with osteoblast-lineage cells, this inhibitory effect has not been observed [69]. On the other hand, osteoclast formation was stimulated by 1,25(OH)2D3 at the same doses in co-cultures of osteoblast lineage cells and hematopoietic cells [70].

Oral administration of vitamin D compounds into mice increased serum calcium via enhanced osteoclast activation. Nevertheless, long-term treatment of mice with pharmacological doses of vitamin D compounds increased trabecular bone volume and mineral density with decreased bone resorption [65, 69]. The ratio of RANKL/OPG expression in bone was decreased by long-term treatment with a pharmacological dose of vitamin D compounds.

The vitamin D receptor-regulated expression of RANKL and OPG in osteoblast-lineage cells may be finely modulated by mineral homeostasis or mineral-regulating hormones such as PTH and FGF23. Collectively, VDR in osteoblast-lineage cells does not play major roles for homeostatic turnover of bone but was essential for increasing trabecular bone volume and mineral density in mice treated with pharmacological doses of vitamin D compounds [68, 71]. Active 1,25(OH)2D3 binds to VDR with high affinity and selectivity. Its expression levels decreased in

mature osteoclasts, and vitamin D may act on VDR-rich osteoclast precursors. A recent study observed that active 1,25(OH)2D3 may inhibit osteoclast differentiation by suppressing a calcium channel protein, TRPV5, expression at the early stage [72]. However, surprisingly, 1a,25-(OH)2D3 also has a positive effect on bone resorption and osteoclastogenesis, which hinders the improvement of osteoporosis to some extent. 1a,25-(OH)2D3 activated osteoclast formation in RANKL-induced RAW264.7 cell [73].

In Japan, calcitriol and its prodrugs, alfacalcidol [1α-hydroxyvitamin D3, 1α (OH)D3], and eldecalcitol [1α,25(OH)2-2β- (3-hydroxypropyloxy)D3], have been used as drugs for the treatment of osteoporosis since these drugs increase the bone mineral density of trabecular bones and decrease the risk of fractures. Paradoxically, the beneficial effects of these vitamin D drugs are caused by the down-regulation of bone resorption [74].

**Vitamin E**

Vitamin E refers to a family of eight naturally-occurring homologs that are synthesized in plants. All tocopherols and tocotrienols that exhibit alpha-tocopherol activity. Vitamin E is the major lipid-soluble antioxidant in the cell and is obtained from the diet. By under the phenolic hydrogen on the 2H-1-benzopyran-6-ol nucleus, these compounds exhibit varying degrees of antioxidant activity, depending on the position and number of methyl groups and the type of isoprenoids. There is also a widely available synthetic form, dl-α-tocopherol, prepared by coupling trimethyl hydroquinone with isophytol. This consists of a mixture of eight stereoisomers in approximately equal amounts [26, 75].

Wong et al. recently reviewed the bone protecting effect of vitamin E. They observed vitamin E and its derivatives exhibited potential protective effects in osteoporotic animals induced by various stressors, such as ovariectomy, orchidectomy, nicotine, alcohol, free radicals, glucocorticoid, buserelin, and metabolic syndrome [75]. In vivo and co-culture experiments suggest that vitamin E suppresses the secretion of RANKL production in osteoblast cells via its antioxidant and anti-inflammatory properties [75-78].

In vitro osteoclast differentiation assay indicated that α-tocotrienols and trolox inhibited osteoclast differentiation by suppressing c-Fos, ERK, and NF-κB activation [76, 78]. In contrast, α-tocopherols exerted stimulation effect osteoclast fusion in vivo and in vitro osteoclast differentiated from the bone marrow-derived cell via activating p38 and MITF [79]. The effect of vitamin E on canonical Wnt/beta-catenin signaling in bone cells has not yet been elucidated; it is a promising area in osteoclast activation. Collectively, the reported positive outcomes of vitamin E on bone indicate the existence of an anti-osteoporotic effect in vitamin E.

**Vitamin K**

Vitamin K is a lipid cofactor required for normal blood clotting. The letter "K" stands for "Koagulation", the Danish term for coagulation. Several forms of vitamin K have been identified: vitamin K1 (phytomenadione) derived from plants, vitamin K2 (menaquinone) from bacteria, and synthetic naphthoquinone provitamins, vitamin K 3 (menadione). Vitamin K3 is water-soluble, and it is converted to vitamin K2 in the liver. Natural sources of Vitamin K are green leafy vegetables, liver, cheese, butter, and egg yolk. Vitamin K enters the cells and functions as the cofactor of the endoplasmic reticulum resident γ-glutamyl carboxylase (GGCX), which carboxylates any selected glutamate residues on the target proteins and enables these proteins to bind to calcium [26].

The effect of vitamin K on bone health recently reviewed by Tsugawa et al. and Akbari et al. Vitamin K is reported to have an inhibitory effect on osteoclastic bone resorption in murine osteogenic culture, rabbit model, and rat models. Vitamin K prevents bone resorption via several mechanisms [80, 81]. It prevents osteoclast formation directly on osteoclast or indirectly interferes with the expression of RANKL and upregulates the expression of OPG on osteoclast precursors [82-84]. Also, vitamin K decreases both proliferation of osteoclast and enzymatic activity in osteogenic culture medium [85, 86]. Moreover, vitamin K2 inhibits bone resorption, induced by bone-resorbing factors such as PGE2, IL1α, and 1, 25(OH) 2D3 in a dose-dependent manner [80, 82]. Vitamin K2 downregulates basal and cytokine-induced NF-κB activation in human and murine monocytic cell lines, and thereupon prevented the osteoclast activation [84, 86]. Taken together, the current evidence suggests that vitamin K reduces osteoclast differentiation and activation.

**Water-soluble vitamins**

**Vitamin C**

Vitamin C is a six-carbon lactone. Its chemical names are ascorbic

acid and ascorbate. Ascorbic acid is an essential nutrient in human diets, and necessary to maintain connective tissue and bone. When there is insufficient dietary vitamin C, humans suffer from the potentially fatal deficiency disease scurvy. Vitamin C is considered an antioxidant. It is found naturally in citrus fruits and many vegetables.

Vitamin C has a controversial effect on osteoclast differentiation, and activation depends on cell collection and differentiation stages. Recent meta-analyses concluded that greater dietary vitamin C intake was associated with higher bone mass density and reduced risk of hip fracture and osteoporosis [87]. In vivo studies observed that vitamin C attenuates bone loss and stimulates bone formation via increasing osteocalcin, RUNX2, BMP-2, decreasing RANKL expression [88-90]. A recent osteoporotic animal model study investigated that Wnt3a/β-catenin/ATF4 pathways are associated with vitamin C effect on osteoporosis [91]. In vitro, treatment with vitamin C caused a 5-fold increase in RANKL expression and induced osteoclasts formation when co-cultured the clonal stromal cell line with mouse bone marrow cells [89]. Also, it has been reported previously that vitamin C is an essential factor for osteoclast differentiation in the co-culture system [92, 93].

Vitamin C at 50 μg/ml significantly increased osteoclast number, size, and nuclear number in primary mouse bone marrow cultures and RAW 264.7 cells. However, late-stage osteoclasts treated with vitamin C in culture initiated cell death [94]. In contrast, vitamin C (same dose) inhibited osteoclast formation from BMM via suppression of RANK expression [95] and reduces the formation of bone resorption pits in vitro [96].

**Vitamin B**

The effects of B vitamins on bone health have been well summarized in two recent reviews. The protective role of vitamin B1, B2, B6, B12, and folic acid in bone health has been recognized [97-99].

Vitamin B complex is considered six water-soluble vitamins, including B1, B2, B6, pantothenic acid, and biotin.

**Vitamin B1** (thiamin, aneurin) is in the B complex family. Thiamine is required for metabolism, including that of glucose, amino acids, and lipids. With specific transporters, thiamine can be transported into mammalian cells and subsequently transformed into thiamine diphosphate (ThDP) by the cytosolic thiamine pyrophosphokinase. After that, ThDP can be phosphorylated to thiamine triphosphate (ThTP). ThDP is regarded as cofactors for several key metabolic enzymes [26].

Thiamine deficiency is common in hospitalized elderly and orthopedic patients, commonly with lower bone mineral density and femoral neck fractures [97, 98]. A recent study demonstrated that vitamin B1 has a protective effect on osteoporotic mice model induced by estrogen deficiency. It also demonstrated vitamin B1 inhibits osteoclast differentiation induced by RANKL in its derivative form of ThDP but not ThTP. The inhibitory effect suppresses intracellular ROS accumulation and unfolded protein response (UPR) signaling via Rac-Nox1/2/4 and IRE1α/XBP1 pathways, respectively [99].

Vitamin B2 (riboflavin) is a coenzyme function in numerous oxidation and reduction reactions. Its deficiency causes cheilosis, angular stomatitis, and dermatitis. Conversion of vitamin B2 to flavin mononucleotide (FMN) and then to the predominant flavin, flavin adenine dinucleotide (FAD), occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. The flavocoenzymes (FMN and FASD) participate in oxidation-reduction reactions in metabolic pathways and energy production via the respiratory chain [26].

**Vitamin B2** increased osteoblast activity, decreased osteoclast numbers, and diminished alveolar bone loss in experimental periodontitis in a ligature-induced rat model [100]. Photodegradation products of riboflavin, lumichrome, administration prevented bone loss in ovariectomized mice by decreasing the osteoclast number in vivo. Also, it suppresses RANKL-induced osteoclast formation and bone resorption via inhibiting NFATc1 activation and calcium signaling [101].

**Niacin** (nicotinic acid and nicotinamide) is a water-soluble vitamin of the B complex occurring in various animal and plant tissues. It is required by the body for the formation of coenzymes NAD and NADP. Its deficiency causes Pellagra with diarrhea, dermatitis, and dementia. An effect of niacin on osteoclasts is not clear.

**Vitamin B5** (pantothenate or pantothenic acid) is a component of CoA, a cofactor that carries acyl groups for many enzymatic processes, and of phosphopantetheine within acyl carrier proteins, a component of the fatty acid synthase complex. The compounds containing pantothenate are most especially involved in fatty acid metabolism, and the pantothenate-containing prosthetic group additionally facilitates binding with appropriate enzymes [26]. Low concentrations of vitamin

B5 induces osteoclast differentiation by stimulating the PI3K-Akt pathway, while a higher concentration of vitamin B5 suppresses osteoclastogenesis by scavenging ROS generation. In vivo, animal studies using the ovariectomized model suggest a potential role of pantothenic acid-rich diet in protecting bone loss against estrogen deficiency [102].

**Vitamin B6** refers to several picolines (especially pyridoxine; pyridoxal; and pyridoxamine) that are efficiently converted by the body to pyridoxal phosphate, which is a coenzyme for the synthesis of amino acids, neurotransmitters (serotonin, norepinephrine), sphingolipids, and aminolevulinic acid. During transamination of amino acids, pyridoxal phosphate is transiently converted into pyridoxamine phosphate [26]. Vitamin B6 deficiency in rats caused a significant delay in the maturation of fracture callus. A recent study demonstrated that deficiency of vitamin B6, vitamin B12, folate, and combination of these three vitamins caused a significant accumulation of homocysteine, accompanied by a distinct stimulation of the resorption activity in osteoclast cell culture [103].

**Vitamin H or biotin.** Dietary deficiency in normal people is probably rare. Some patients have multiple carboxylase deficiencies, and there are occasional biotinidase deficiencies. Clinical signs of deficiency include dermatitis of an erythematous and seborrheic type, conjunctivitis, alopecia, and central nervous system abnormalities such as hypotonia, lethargy, and developmental delay in infants, and depression, hallucinations, and paresthesia of the extremities in adults. Biotin functions as a coenzyme within several carboxylases. In humans, biotin operates within four carboxylases [26]. The effect of biotin on osteoclast is not clear.

**Vitamin B9 or folic acid** (folates) accept one-carbon units from donor molecules and pass them on via various biosynthetic reactions. In their reduced form, cellular folates function conjugated to a polyglutamate chain. These folates are a mixture of unsubstituted polyglutamyl tetrahydrofolates and various substituted one carbon forms of tetrahydrofolate. The reduced forms of the vitamin, particularly the unsubstituted dihydro and tetrahydro forms, are unstable chemically. They are easily split between the C-9 and N-10 bond to yield a substituted pteridine and p-aminobenzoylglutamate, which have no biologic activity. Substituting a carbon group at N-5 or N-10 decreases the molecule's tendency to split; however, the substituted forms are also susceptible to oxidative chemical rearrangements and, consequently, loss of activity. The folates found in food consist of a mixture of reduced folate polyglutamate. The chemical lability of all naturally-occurring folates results in a significant loss of biochemical activity during harvesting, storage, processing, and preparation [26].

Several epidemiologic studies have found a significant relationship between increased folic acid intake and increased reduced fracture risk [97]. Daily supplementation of folic acid during pregnancy could positively impact the bone turnover markers and osteoclast activation markers in mothers and their newborns [104]. Folic acid prevents bone loss via decreased osteoclast number in rats' experimental models such as cyclosporine-induced bone loss and periodontal disease model [100, 105].

In the folate-deficient treated osteoclasts, resorption activity was significantly increased compared to the folate treated cells. Furthermore, low folic acid, B12, and B6 concentrations in osteoclasts resulted in the accumulation of homocysteine and stimulation of resorption activity of osteoclasts in vitro but did not influence the activity of human osteoblasts in vitro [106]. Alternatively, folic acid may help maintain bone density by helping to preserve optimal nitric oxide synthase activity in bone cells [107].

**Vitamin B12** (cobalamin) is the largest of the B complex vitamins, with a relative molecular mass of over 1000. It consists of a corrin ring made up of four pyrroles with cobalt at the center of the ring. A recent meta-analysis of observational studies has found that elevated homocysteine levels and low vitamin B12 and folate levels have been associated with structural deterioration of bone tissue. Epidemiological cohort studies show strong associations between low levels of Vitamin B12 and homocysteine serum concentrations and a high incidence of fractures. Vitamin B12 deficiency in a genetic mouse model results in osteoporosis [97]. A recent study demonstrated that deficiency of vitamins B12 and combination with B6 and folic acid caused a significant accumulation of homocysteine, accompanied by a distinct stimulation of the resorption activity in osteoclast cell culture [103]. The direct effect of vitamin B12 on osteoclast formation and activation is still unclear.

**Table 1.** Effect of vitamins on osteoclastogenic signaling molecules.

| Vitamin | Cell | Target molecules | Effect on osteoclast differentiation | Reference |
|---|---|---|---|---|
| Vitamin A | RAW264.7 | RANK↓ | inhibits | [66] |
| | | c-fos↓ | | |
| Retinaldehyde | BMM | PPARγ↓ | inhibits | [63] |
| 1,25(OH)2D3 | BMM | TRPV5↓ | inhibits | [72] |
| 1,25(OH)2D3 | RAW264.7 | c-Fos↑ | activates | [73] |
| | | NFATc1↑ | | |
| α-tocotrienolsa | BMM | c-Fos ↓ | suppresses | [76] |
| | | ERK ↓ | | |
| | | NF-κB ↓ | | |
| α-tocopherol | BMM | DC-STAMP | stimulates osteoclast fusion | [79] |
| | | P38 ↑ | | |
| | | Mitf ↑ | | |
| Troloxb | BMM | c-Fos ↓ | inhibits | [78] |
| Vitamin K2 | RAW264.7 | NF-κB ↓ | inhibits | [84] |
| Vitamin K1c | BMM | c-Fos↓ | inhibits | [86] |
| | | NFATc1↓ | | |
| Vitamin C | RAW 264.7 | oxidative stress | activates | [94] |
| | BMM | | | |
| Vitamin C | BMM | RANK↓ | inhibits | [95] |
| | | c-fos↓ | | |
| B2 Lumichrome | BMM | NFATc1↓ | inhibits | [101] |
| Vitamin B5 | BMM | | | [102] |
| <200 μM | | PI3K-Akt↑ | activates | |
| >500 μM | | ROS↓ | inhibits | |
| Vitamin B1d | BMM | c-fos↓ | inhibits | [99] |
| | | Rac-Nox1/2/4↓ | | |
| | | IRE1α/XBP1↓ | | |
| | | NFATc1↓ | | |

[a]But not α-tocopherols, [b]A water-soluble vitamin E analogue, [c]MK-4 and MK-7, [d]ThDP but not ThTP

## Discussion

This review focused on the positive and negative regulatory effect of vitamins on osteoclast differentiation in vivo and in vitro, especially in signal transduction. Skeletal remodeling depends on the orchestrated interplay between bone formation by osteoblasts and bone resorption by osteoclasts. Osteoclasts develop in an environment populated by several other cell lineages and rely on these cell's signals to develop and function. As discussed previously, osteoblasts provide the signals for osteoclastogenesis in the form of secreted RANKL. Moreover, it is increasingly evident that other cells, such as immune cells, play a critical role in osteoclast differentiation. and as osteoclasts and their precursors are sensitive to pro- and anti-inflammatory cytokines signals.

Disbalance of bone cell interaction and over activation of osteoclasts cause osteoporosis and subsequently increase bone fracture risk. The 2000 National Institutes of Health (NIH) consensus meeting defines osteoporosis as a skeletal disorder characterized by compromised bone strength predisposing to increased fracture risk. The bone mineral content and its spatial orientation determines bone strength and bone quality and declines with bone construction abnormalities, such as deterioration of bone matrix proteins and bone microarchitecture,

high or low bone turnover rate, and accumulation of microdamage. These abnormalities are further complicated as a result of biological senescence, which is associated with vitamin deficiencies.

The anti-osteoporotic role of vitamin D, E, K, C, B1, B2, B6, B12, and folic acid by inhibiting osteoclast activation has been recognized [74, 75, 80, 88, 97-99]. In contrast, vitamin A enhances the osteoclast resorption activity in vivo, and it has an inhibitory effect on vitamin D function [65]. Furthermore, vitamin A has site-specific effects. It enhances activation of osteoclasts on the periosteal surface of cortical bone, whereas it reduces the osteoclasts formation in the bone marrow in trabecular bone and on endosteal surfaces [67].

Vitamins have a broad effect on bone metabolism. Vitamin A, D, E, and C act on both osteoclasts and osteoblasts. The effects of these vitamins on osteoclast differentiation and activation differ in vitro osteoclast culture or co-culture with osteoblast and in vivo. Vitamin A inhibits RANKL-induced osteoclast differentiation but enhances resorption in co-culture systems and in vivo animal models [67]. Vitamin D regulates osteoblasts' physiological activity by enhancing RANKL secretion, which enhances the differentiation of osteoclast. On the other hand, vitamin D can also inhibit c-Fms and RANK expression in osteoclast precursor and, therefore, inhibit osteoclast differentiation. In vivo studies observed vitamin E and its derivatives exhibited potential protective effects in osteoporotic animals induced by various stressors, such as ovariectomy, orchidectomy, nicotine, alcohol, free radicals, glucocorticoid, buserelin, and metabolic syndrome [75]. In vivo and co-culture experiments suggest that vitamin E suppresses the secretion of RANKL production in osteoblast cells [75-78]. Vitamin E inhibits osteoclast differentiation in vitro [76, 78] but stimulates osteoclast fusion in vivo and in vitro mature osteoclast culture [79]. Vitamin C has a controversial effect on osteoclast differentiation and activation, depending on cell collection and differentiation stages. Collectively, vitamin C has an anti-osteoporotic effect, but an exact mechanism remains unclear.

Current evidence from experimental studies provides several mechanistic pathways for B6, folate, and B12 on bone physiology. A recent study demonstrated that deficiency of vitamin B6, vitamin B12, folate, and combination of these three vitamins caused a significant accumulation of homocysteine, accompanied by a distinct stimulation of osteoclast resorption activity in cell culture [103].

The inhibitory effect of vitamin K and vitamin B2 on osteoclast differentiation and activation is thought to be caused by inhibition of NF-κB following the suppression of NFATc1. Their anti-osteoporotic effect is observed in vivo and preclinical studies [84, 86, 101]. The present review has two limitations. First, we focused only on osteoclast differentiation and function, not osteoblast function, a significant regulator of bone resorption in vivo. Second, vitamin function is associated with minerals, and some hormones, such as vitamin D's action on bone resorption, correlate with calcium level. A trace mineral belongs to a micronutrient as vitamins. Further, the regulatory effect of trace minerals on osteoclast differentiation and function should be reviewed.

In conclusion, Vitamin D, E, B1, B5, B6, and B12 have mainly anti-osteoporotic effects; however, their mechanism on osteoclast differentiation and activation is variable. Vitamin A and C have been considered to activate osteoclast differentiation and function, but some report a suppressive effect on osteoclast function. Vitamin K and B2 exert an inhibitory effect on osteoclast differentiation and activation both in vitro and in vivo. While niacin, biotin, and folic acid have a direct effect on osteoclast differentiation, and their effect on activation remains unclear. Collectively, vitamins act on osteoclast differentiation and function in various ways depending on cell type, cell maturation and microenvironment.

## Conflict of Interest

The authors declare that they have no competing interests.

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