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The role and therapeutic potential of vitamins in fracture healing

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Abstract：

Fracture healing is an energetically demanding, highly orchestrated process in which inflammation, angiogenesis, callus formation and remodelling must be tightly coordinated, yet up to 10% of fractures progress to delayed union or non-union. Emerging evidence positions vitamins as key modulators of osteoimmunology, cellular metabolism and matrix mineralisation across distinct phases of repair. In this Review, we

first outline the cellular and metabolic landscape of fracture healing, highlighting the roles of osteoblasts, osteoclasts, osteocytes, bone-lining cells and mesenchymal progenitors, and the coupling of bone and immune cells. We then synthesise data on vitamins A, C, E, K and B in bone health and fracture repair. Physiological retinoid signalling supports early osteoblast differentiation, whereas both deficiency and chronic excess of vitamin A drive osteoclastogenesis, disturb FGF23–vitamin D–Wnt axes and impair mineralisation in a dose-, time- and site-dependent manner. Vitamin C, as a cofactor for collagen crosslinking and a potent antioxidant, promotes osteoblast and mesenchymal stem-cell differentiation, modulates osteoclast survival and may mitigate oxidative blockade of callus formation. Vitamin E, particularly tocotrienols, limits oxidative stress, suppresses RANKL-driven osteoclastogenesis and activates Wnt/β-catenin and BMP pathways to favour bone formation. Vitamin K integrates γ-carboxylation of bone vitamin K–dependent proteins with PXR/SXR- and mitochondria-mediated signalling to support proper calcification and restrain excessive resorption. B vitamins regulate fracture healing through the one-carbon metabolism–homocysteine and NAD^+–sirtuin axes, influencing epigenetic control of osteogenic genes, collagen crosslinking and skeletal stem-cell function. Finally, we discuss synergistic and antagonistic interactions among vitamins (for example, vitamins D–K and C–E versus excess vitamin A or α-tocopherol), and propose a stage-specific, biomarker-guided, minimal-effective-dose strategy for personalised vitamin-based adjuncts to fracture care.

Keyword：Fracture Healing, Vitamin, Bone health

1 Introduction

A fracture is a disruption of bone continuity that may be associated with injury to adjacent blood vessels, nerves, soft tissues, or organs. Globally, about 34% of men and nearly 50% of women will experience a fracture in their lifetime. Fracture healing aims to restore bone mechanical integrity and function, influenced by factors like health, nutrition, age, hormones, signaling molecules, and mechanical stress[1].At the cellular level, fracture repair is driven by the coordinated proliferation, differentiation and crosstalk of mesenchymal progenitors, osteoblasts, osteoclasts, osteocytes and immune cells.While most fractures heal successfully, around 10% of patients experience complications such as delayed union or nonunion[2].Delayed fracture healing and complications, both early complications (such as hemorrhagic shock, wound infection, and nerve injury) and late complications (such as pneumonia, pressure ulcers, and post-traumatic osteoarthritis), significantly impact patients' quality of life, finances, and mental health, imposing a substantial socioeconomic burden. Thus, effectively managing fractures and their complications is crucial in skeletal health care.Vitamins are crucial for maintaining the body's metabolic and organ balance and are known to regulate bone metabolism. While many studies have examined how vitamins affect bone cells, their role in fracture healing hasn't been comprehensively reviewed. This article

examines how vitamins A, C, E, K, and B contribute to fracture healing and interact with each other. It also suggests new insights to enhance fracture treatment and guide future clinical practices.

2 Fracture healing process

Clinically, fracture healing is generally divided into four stages: the inflammatory phase with hematoma formation, the phase of soft callus formation, the phase of hard callus formation, and the remodeling phase. These stages are not discrete; rather, they form an interconnected continuum both temporally and spatially. This interconnection involves the coupling of bone and blood vessels, the integration of immune responses with bone metabolism, and the differentiation of bone cells[3, 4]. The duration of each stage may vary based on factors such as the fracture's location and type, the extent of vascular and periosteal damage, and the individual's metabolic status. The fracture healing process is influenced by a multitude of factors, including inflammatory mediators, mechanical signals, hypoxic conditions, aging, pharmacological effects, and lifestyle-related factors of the patient[5-9].From a microscopic perspective, the process of fracture healing is distinguished by a sophisticated interaction among various cellular components and metabolic factors. In the initial phase, the earliest cellular response to a fracture is marked by the presence of inflammatory cells, such as T cells, B cells, mast cells, macrophages, eosinophils, and neutrophils[10]. Mesenchymal progenitor cells, endothelial

cells, chondrocytes, and osteoblasts initiate bone formation, followed by a remodeling phase controlled by the balance between osteoblasts and osteoclasts, all working together to heal fractures[11].

From the perspective of the various stages of fracture healing, the initial phase, known as the hematoma inflammation and organization phase, typically spans from several hours to several weeks. This phase is primarily characterized by the formation of a hypoxic, fibrin-rich provisional matrix, resulting from associated soft-tissue injury and hematoma associated with the fracture. The predominant cellular components during this phase are neutrophils and subsequently infiltrating monocytes/macrophages. The release of inflammatory factors like TNF-α, IL-1, IL-6, and DAMPs aids in recruiting mesenchymal stem cells and their transformation into chondrocytes and osteoblasts[12]. Initially, the M1 macrophage phenotype is primarily involved in mediating injury clearance, whereas later polarization towards the M2 phenotype predominantly supports tissue repair[13]. The SDF-1/CXCR4 axis, along with PDGF and FGF, guides MSC/SSC homing to fracture site[14].The transition from M1 to M2 macrophage polarization plays a crucial role in shifting the physiological response from inflammation to tissue regeneration. In cases of excessive or prolonged inflammation, commonly observed in multiple injuries, there is a tendency for fibrous nonunion to occur[15]. Hypoxia-inducible factor 1-alpha (HIF-1α) becomes stabilized in hypoxic hematomas, leading to the direct upregulation of vascular endothelial growth factor A (VEGFA) and promoting subsequent

vascularization[16]. Within a two-week period, the reparative process progresses to the soft callus formation phase, during which HIF-1α, under hypoxic conditions, induces VEGF production and initiates glycolysis[17], thereby enhancing angiogenesis, osteogenic differentiation, and their coupling. Simultaneously, M2 macrophages release TGF-β, VEGF, and exosomes to promote angiogenesis and bone formation[18]. During this phase, cartilage matrix components such as collagen type II alpha 1 (COL2A1) and aggrecan (ACAN) are deposited, and hypertrophic chondrocytes emerge. Importantly, some of these hypertrophic chondrocytes have the potential to differentiate into osteoblasts and osteocytes[19].Two weeks subsequent to the initial phase, the process progresses to the formation stage of the primary bone callus. This stage is regulated by critical signaling pathways, including BMP2/7–Smad, Wnt/β-catenin, and Ihh–PTHrP, which facilitate the transformation of chondrocytes into bone and promote intramembranous ossification[20]. Concurrently, the YAP/TAZ signaling pathway converts mechanical stimuli into transcriptional programs that support vascular invasion and the expansion of osteogenic lineages[21], while Piezo1 is implicated in the sensing of mechanical load and the osteogenic response[22]. type H endothelial cells are found in conjunction with proliferating osteoprogenitors, thereby linking angiogenesis with osteogenesis[21]. Within the hypertrophic cartilage zone, there is expression of COL10A1, ALP, and MMP-13, with MMP-13 playing a crucial role in matrix degradation and the

formation of channels for vascular invasion; a deficiency in MMP-13 significantly impedes cartilage resorption and ossification. Additionally, hypertrophic chondrocytes release VEGF and express RANKL, which are essential for the recruitment and activation of osteoclasts and chondroclasts necessary for the resorption of mineralized cartilage[23].Following the aforementioned process, the fracture undergoes substantial healing and subsequently transitions into the fracture callus remodeling and shaping phase, which may extend from several months to several years. During this phase, vascularized woven bone is progressively replaced by lamellar bone. This transformation is orchestrated by osteoclast-osteoblast coupled signaling pathways, including the RANKL/OPG ratio, TGF-β release, and bidirectional signals such as EphrinB2-EphB4, which collectively regulate osteoblastic refilling post-resorption[15, 24, 25]. fracture callus remodeling, representing the final stage of fracture healing at the macroscopic level, can be succinctly described as the replacement of degraded woven bone with mature and structurally robust lamellar bone[26]. A critical component of this process is the activity of osteoclasts and osteoblasts, alongside the metabolic regulatory network that governs the quantity and activity of osteoclasts[27]. Osteoclasts adhere tightly to their resorption zone on the bone surface designated for remodeling and secrete lysosomal proteins into this sealed area via vesicles, thereby facilitating the digestion of the bone matrix collagen[28].The degradation of bone-related tissues mediated by osteoclasts results in the release of numerous ligand molecules, including

TGF-β, complement 3a, Wnt10b, BMP 6, and SLIT 3, which play a significant role in facilitating subsequent lamellar bone formation and replacement processes[29, 30]. Furthermore, following the inflammatory phase of a fracture, small bone fragments can recruit osteoclasts, leading to resorption at the fragment edges. These fragments, after undergoing selection, integrate into the overall callus, indicating their regulatory role during callus formation[31]. The remodeling function of osteoclasts enhances bone density and strength[32]. During metabolism, various cytokines, metabolites, and other factors can influence the fracture healing process by affecting the differentiation, activity, and number of osteoclasts[33, 34].

3 Key Role of Cellular Metabolism in Fracture Healing

Fracture healing is orchestrated by a multitude of signaling pathways and entails complex interactions among various cell types within the bone marrow. The differentiation and communication of these diverse cell types at distinct stages of fracture healing establish a precisely regulated network essential for the comprehensive repair of the fracture.

3.1 Osteoblasts

The characteristic structures of osteoblasts, predominantly located along the bone surface, encompass approximately three nucleoli, an abundance of rough endoplasmic reticulum and Golgi apparatus, secretory vesicles, and an extracellular matrix enriched with type I collagen. These

structural features facilitate the fulfillment of high osteogenic demands and contribute to cellular stability[35]. Osteoblasts secrete osteoid into the bone matrix and enhance the synthesis of matrix-associated proteins. Osteogenic differentiation is intricately regulated by a multitude of intracellular and extracellular factors, with osterix and RUNX2 serving as primary regulators—RUNX2 acts upstream to modulate osterix. Additional transcription factors involved include ATF4, SATB2, and TAZ/YAP[36, 37]. Osteogenic differentiation is associated with several signaling pathways, including Wnt, BMP, TGF-β, Hedgehog, PTH, FGF, Ephrin, Notch, Hippo, and Piezo1/2[36, 38-42].Osteoblast-lineage cells, including bone-lining cells, secrete matrix metalloproteinases that remove unmineralised osteoid and expose the mineralised surface, thereby initiating the activation phase of bone remodelling. The synthesis of the bone matrix by osteoblasts can be delineated into two distinct phases. In the initial phase, osteoblasts secrete collagen, non-collagenous proteins, core proteoglycans, glycosaminoglycans, and additional proteoglycans. The subsequent phase involves mineralization, which is accomplished through both vesicular and fibrous processes[43]. In the vesicular phase, osteoblasts release 30-200 nm matrix vesicles that bind to proteoglycans. Sulfated proteoglycans cause calcium ion denaturation, and after enzymatic breakdown, calcium ions enter the vesicles through annexin channels[44]. Alkaline phosphatase (ALP) degrades phosphorylated compounds to release phosphate, and together with calcium, they form hydroxyapatite (HA) crystals within the vesicles. In the fibrous phase, the

vesicles rupture due to calcium-phosphate supersaturation, allowing HA crystals to diffuse into the surrounding matrix[45]. After the bone matrix matures, osteoblasts mainly follow one of three paths: they either embed in the matrix to become osteocytes, turn into inactive bone lining cells on the surface, or degenerate[46].

3.2 Osteoclasts

Osteoclasts, originating from the monocyte lineage of hematopoietic stem cells within the bone marrow, are the sole cellular entities in the body capable of actively resorbing bone tissue. These multinucleated giant cells exhibit a high metabolic state, characterized by their ruffled border and clear zone, which serve as the principal sites for bone resorption. Osteoclasts adhere to the bone surface via specific integrins[47] and degrade bone and cartilage matrices via secretion of acidic hydrolases and proteases, including cathepsin K and matrix metalloproteinases, together with high activity of the lysosomal enzyme tartrate-resistant acid phosphatase (TRAP). Osteoclast metabolism and development are mainly controlled by osteoblast lineage and stromal cell factors, including M-CSF and RANKL[48]. Fluid shear stress (FSS) plays a crucial role in regulating osteoclast proliferation, differentiation, and function through various downstream signaling pathways. These pathways include the calcium-mediated TRPV4 and STIM pathways, the ERK5 pathway, as well as ATP6V1 and TCIRG1[49-52].

3.3 Bone-lining cells

During the process of fracture healing, bone lining cells (BLCs) function not merely as "passive barriers" covering the surface of quiescent bone, but rather as a crucial osteogenic reservoir integral to tissue repair and bone regeneration, as well as a central hub for bone remodeling[53-55]. Foundational studies have demonstrated that bone lining cells possess the capability to clear residual collagen and matrix fragments from the Howship lacunae during the reversal phase following bone resorption. This activity creates a microenvironment that is conducive to subsequent new bone deposition and directly initiates the bone formation process[53, 54].Subsequent research has corroborated that quiescent periosteal cells within adult bone tissue can be reactivated and redifferentiated into functional osteoblasts. This finding implies that these cells play a crucial role in the formation and remodeling of new bone following fractures, particularly on the surfaces of the inner periosteum and trabecular bone[55, 56]. Mechanistically, periosteal cells exert their influence primarily through a continuous process of "activation—expansion—osteogenesis—coupled remodeling"[57-59]. On one hand, parathyroid hormone (PTH) and its receptor (PTH1R) signaling rapidly induce quiescent periosteal cells to transition from a dormant state to active osteoblasts. This process is synergistically enhanced by the Wnt/β-catenin signaling pathway, which promotes osteogenic programs such as RUNX2 and osteocalcin (OCN). Additionally, mechanical stimulation can activate periosteal cells and their associated osteocyte networks, thereby promoting bone formation through

mechanisms including the release of SOST/Dkk1 inhibition and the activation of β-catenin[60-63].Conversely, periosteal cells are integral to the coordinated regulation of bone resorption and formation, with their phenotype being intricately linked to bone remodeling molecular networks such as RANKL/OPG and M-CSF. This association influences osteoclast recruitment, surface remodeling post-resorption, and the initiation of osteogenesis[64-67] Simultaneously, classical osteogenic signaling pathways, including BMP/Smad, Hedgehog, and Notch, intersect with these networks, collectively determining the transition of periosteal cells to an osteogenic phenotype and influencing the quality of callus maturation[56, 68-70]. The fundamental significance of periosteal cells in fracture healing is attributed to their role as a source of local osteogenesis and their mediation of bone remodeling processes. Thus, periosteal cells are regarded as crucial potential targets for enhancing fracture repair and addressing delayed healing[56, 58, 60, 71].

3.4 Osteocytes

Osteocytes constitute the principal and most persistent cellular component of bone tissue, accounting for approximately 95% of the entire bone cell population. These cells primarily originate from osteoblasts, which are derived from mesenchymal stem cells (MSCs), and undergo differentiation through four distinct stages: osteoblasts, pre-osteocytes, young osteocytes, and mature osteocytes[46]. The differentiation from

osteoblasts to osteocytes is characterized by several key changes: an increased nuclear-to-cytoplasmic ratio indicative of diminished protein synthesis and secretion, a reduction in organelle numbers, and alterations in cell morphology. As osteocytes mature and become embedded within the mineralized bone matrix, there is a notable decrease in the expression of osteoblast-specific markers, while the expression of DMP1 and SOST is significantly upregulated[72, 73].Osteocytes establish connections with adjacent osteocytes, osteoblasts, and osteoid surface cells via cytoplasmic extensions within the osteocyte lacuna-canalicular system. This network facilitates the transfer and exchange of substances and signaling molecules, such as bone morphogenetic proteins (BMPs), Wnt proteins, prostaglandin E_2 (PGE_2), and nitric oxide (NO), among cells[74, 75]. Furthermore, the system functions as a mechanosensor among various bone tissue cells, playing an essential role in mechanotransduction pathways[76]. Furthermore, osteocytes serve as the principal source of osteoprotegerin (OPG), playing a critical role in modulating the equilibrium between osteogenesis and osteoclastogenesis by regulating the expression of OPG and receptor activator of nuclear factor kappa-B ligand (RANKL)[77].

4 Key Vitamins'Role in Bone Health and Fracture Prevention

Vitamins are essential nutrients the body needs but cannot produce in sufficient amounts, requiring dietary intake. They are crucial for growth,

metabolism, immunity, and cellular function. Research highlights their importance in maintaining bodily balance and their growing relevance in bone health. This discussion will explore the molecular roles of key vitamins in bone health and fracture healing, aiming to offer new nutritional support strategies for patients with fractures.

4.1 Vitamin A

Vitamin A is a fat-soluble micronutrient predominantly stored in the liver, where it plays a crucial role in regulating various cellular differentiation processes and maintaining metabolic homeostasis[78, 79]. As it cannot be synthesized endogenously in the human body, it must be acquired through dietary sources[80]. Vitamin A exists in two primary forms: the first is animal-derived retinol, which binds to cellular retinol-binding protein 2 (CRBP 2) in the intestine, where it is acetylated into retinyl esters. These esters are then transported via chylomicrons into the bloodstream through the lymphatic system[81, 82] or enter the liver, where they undergo enzymatic hydrolysis before binding to cellular retinol-binding protein 1 (CRBP 1). Subsequently, retinol either associates with retinol-binding protein 4 (RBP 4) or undergoes esterification in adipose tissue[83, 84]. The second form is plant-derived provitamin A, which includes compounds such as α-carotene, β-carotene, and β-cryptoxanthin.In human circulation, vitamin A is transported by carrier proteins, such as retinoic acid-binding protein[85, 86]. The biological effects of vitamin A are predominantly facilitated through its

metabolic derivative, all-trans retinoic acid (ATRA), a natural metabolite of retinol. ATRA acts as a ligand that activates nuclear receptors, subsequently modulating the expression of over 500 genes across diverse cell types[78, 87-89]. Vitamin A is essential for maintaining human metabolism and homeostasis. This discussion will provide a detailed examination of the dual effects of vitamin A and its derivatives on bone health and fracture healing, with a focus on their dose-dependent nature. Furthermore, we will explore the potential benefits of vitamin A supplementation in the context of fracture healing. In assessing the role of vitamin A in bone health and fracture risk, two distinct aspects require attention. Firstly, we will analyze the relationship between vitamin A and its derivatives and fracture incidence. Previous epidemiological studies have yielded varying results: some suggest that vitamin A intake is associated with increased bone density and strength, indicating a reduced risk of fractures[90], whereas others associate vitamin A intake with heightened fracture risk and accelerated age-related bone loss[90-92]. Additionally, certain studies report no significant link between vitamin A and fracture occurrence[93]. Vitamin A deficiency has been shown to increase susceptibility to bone loss and osteoporosis in women aged 65 and older[94, 95]. In children, adequate vitamin A intake and maintaining retinol concentrations above 1.24 μmol/L have a positive impact on skeletal growth and development[96]. The inconsistent conclusions may result from differences in vitamin A intake, which affect bone density by altering the balance between bone resorption

and formation. Additionally, retinoic acid and 1,25-dihydroxyvitamin D interact with their respective receptors, RAR and VDR, and through the shared RXR receptor, they regulate bone calcium levels, influencing mineralization[97, 98]. The association between vitamin A intake and bone health is contingent upon individual variability, manifesting as either beneficial or detrimental. For instance, elevated vitamin A consumption has been correlated with a heightened risk of fractures among individuals with overweight conditions[99]. In Chinese men and women, dietary intake or serum levels of vitamin A are positively correlated with bone mineral density at various anatomical sites[100].Karamati et al. demonstrated a positive correlation between the intake of vitamin A and beta-carotene and lumbar bone mineral density in postmenopausal Iranian women[101]. However, an increase in dietary vitamin A intake has been associated with an elevated risk of spinal and hip fractures[102, 103] and a reduction in vitamin D levels[104]. The differential effects of vitamin A on bone health may be modulated by individual variability, gender differences, dose-dependent responses, and site-specific factors（Fig.1）.

From a molecular biology standpoint, sufficient levels of vitamin A, along with its precursors and derivatives, can enhance osteoblast activity[105], inhibit osteoclast activity[106], and suppress osteocyte differentiation, thereby promoting bone strength and health[107]. Circulating retinol is mainly transported bound to retinol-binding protein 4 (RBP4) and taken up by cells via STRA6, a membrane receptor that mediates

bidirectional retinol transport between extracellular RBP4 and intracellular retinoid-binding proteins. Postprandial retinyl esters are additionally delivered to tissues in chylomicrons via the lymphatic system. Research indicates a significant upregulation of STRA6 expression in mesenchymal stem cells derived from patients with osteoporosis[108]. Consequently, elevated levels of vitamin A are considered a risk factor for osteoporosis[109]. Furthermore, vitamin A is transported to osteoblasts via chylomicrons, converted into trans-retinoic acid, and subsequently binds to the RAR–RXR heterodimer to exert its biological effects[109, 110].Studies show that a 60 μg/g dose of vitamin A acetate in C57 mice reduces bone cortical thickness, indicated by declines in cortical thickness, periosteal and endocortical perimeters, marrow and total areas, osteocalcin, and alkaline phosphatase levels[91, 111]. Conversely, a brief administration of 450 μg/g resulted in a transient enhancement of osteoclast activity, as evidenced by increased levels of acid phosphatase 5 (ACP 5), cathepsin K (CTSK), receptor activator of nuclear factor kappa-B ligand (RANKL), and tartrate-resistant acid phosphatase (TRAP). Simultaneously, a reduction in the concentrations of vitamins D and E was noted[112]. Further studies have shown that treating MC3T3-E1 osteoblasts with retinoic acid concentrations ranging from 1 to 100 μM enhances differentiation by upregulating ALP activity and osteopontin (OPN) expression[113]. Excessive concentrations of retinoic acid (vitamin A) markedly suppressed the proliferation of cultured MC3T3-E1 cells. This suppression was correlated with a downregulation of genes

associated with osteogenesis, such as alkaline phosphatase (ALP), osteocalcin (OCN), Runt-related transcription factor 2 (Runx-2), and nestin (NSX)[113].High-dose vitamin A upregulates FGF23 expression via RAR-dependent mechanisms, which in turn suppresses renal 1α-hydroxylase activity, lowers circulating 1,25-dihydroxyvitamin D, and ultimately impairs bone mineralisation[114, 115].Vitamin A upregulates SOST, a potent inhibitor of canonical Wnt/β-catenin signalling, and modulates PHEX and other regulators of phosphate and vitamin D metabolism[116].Through these pathways, excessive retinoid signalling suppresses Wnt-driven osteoblast activity and impairs bone mineralisation[107]. Additionally, high concentrations of vitamin A may inhibit the formation of mineralized nodules via NF-κB signaling pathways[117]. Concurrent research indicates that provitamin A compounds, such as β-carotene and β-cryptoxanthin, when administered to RANKL-stimulated bone marrow-derived monocytes/macrophages or RAW264.7 cells, reduce cell viability, decrease TRAP-positive area density and other osteoclast-related indicators, increase LDH release (indicative of cell death), and inhibit osteoclastogenesis by affecting NFATc1, c-Fos, and CTSK expression through attenuated NF-κB activation[107, 118]. Excessive consumption of vitamin A leads to the accumulation of its metabolite, retinoic acid, which promotes receptor activator of nuclear factor κB ligand (RANKL)-mediated osteoclast differentiation, consequently facilitating bone resorption[119, 120]. Research involving animal models has shown that administering high doses of vitamin

A, either orally or subcutaneously, leads to a reduction in cortical bone thickness, periosteal bone, and bone strength in mice. This bone loss is associated with an increase in periosteal osteoclasts and elevated levels of urinary hydroxyproline and serum tartrate-resistant acid phosphatase 5b (TRAP 5b), indicative of heightened bone resorption. Mechanistically, the all-trans retinoic acid (ATRA)-induced increase in the RANKL/osteoprotegerin (OPG) ratio further promotes bone resorption. Furthermore, excessive vitamin A intake induces osteoclast-mediated bone resorption and stimulates the overexpression of vascular endothelial growth factor, thereby elevating fracture risk[121]. Studies also suggest that vitamin A may play a role in the regulation of osteoblasts in response to mechanical stress[111]. Moreover, TTR and BCO1 are linked to fasting serum retinol levels. Vitamin A deficiency leads to notable skeletal abnormalities and unusual cortical bone remodeling[122]. Furthermore, the impact of vitamin A on bone health demonstrates temporal variability: moderate intake over a short duration promotes bone anabolism, while prolonged excessive consumption leads to a predominance of bone catabolism[123].

Overall, physiological retinoid signalling appears to promote early osteoblast differentiation, whereas both deficiency and chronic excess—particularly at pharmacological doses—impair bone mineralisation and can lead to skeletal abnormalities. Thus, dose, duration and developmental timing are critical. In contrast, high-dose vitamin A results in the suppression of genes associated with osteogenesis and the activation of

genes related to osteoclastogenesis, thereby inhibiting both osteoblast differentiation and bone mineralization. Consequently, both vitamin A deficiency and excess are detrimental to skeletal health and fracture healing. The observed significant U-shaped relationship between plasma retinol concentration and bone mineral density (BMD) further corroborates these findings[96, 124, 125]. Furthermore, the effects of vitamin A on bone demonstrate distinct site-specificity and individual variability.Consequently, the application of vitamin A, its derivatives, and related formulations in the context of fracture healing and bone health necessitates individualized assessment. Specifically, for fractures, the appropriate dosage of vitamin A should be tailored according to the fracture's anatomical site. Current research is inadequate for the effective translation of findings into clinical practice. Therefore, future investigations in this domain will be essential for enhancing clinical outcomes in fracture healing and fostering bone health, particularly within the elderly demographic.

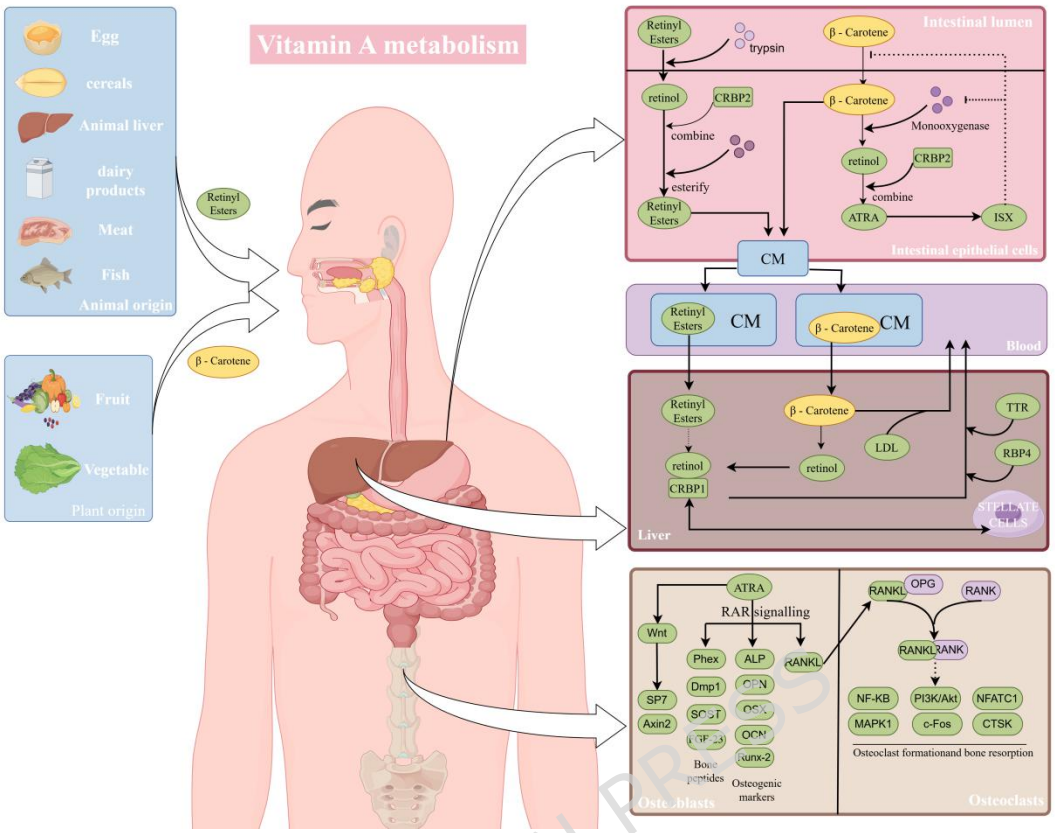

Figure.1:Vitamin A Metabolism and Its Regulatory Effects on Bone

4.2 Vitamin C

Vitamin C, a water-soluble vitamin, primarily exerts its functions in the human body through its antioxidant and tissue-repairing properties. It is notably recognized for its role in the prevention of scurvy. Additionally, vitamin C is involved in critical physiological processes such as collagen synthesis[126], immune system regulation, and iron absorption, predominantly in the form of ascorbic acid (L-AA). As an essential cofactor for the amino acids proline and lysine, ascorbic acid influences the formation of hydroxyapatite crystals and subsequent mineralization by

regulating collagen production, thereby playing a crucial role in bone stability and repair. Research suggests a complex and significant relationship between vitamin C intake and bone density[127], as well as a reduction in apoptosis levels in bone-associated cells[128].As an ascorbic acid derivative, ascorbic acid 2-phosphate significantly enhances the proliferation of human osteoblasts and mesenchymal stem cells, as well as the intracellular mRNA expression levels of type III collagen[129]. In humans, endogenous vitamin C synthesis is not possible because of the lack of L-gulonolactone oxidase (GULO)[126].therefore, it must be acquired through dietary sources. Plant-based foods, such as lemons, oranges, strawberries, and tomatoes, are vital sources of ascorbic acid (vitamin C), whereas animal-based foods generally contain lower concentrations. The bioavailability of vitamin C is contingent upon its intestinal absorption rate and mechanisms, primarily through passive diffusion across the oral mucosa and active transport via the sodium-dependent vitamin C transporter (SVCT) in the gastrointestinal tract[130]. Regardless of whether vitamin C is ingested through food or supplements, it undergoes renal metabolism, with the majority being excreted from the body. Ascorbic acid initially undergoes filtration through Bowman's capsule before being reabsorbed by transport proteins in the proximal tubule. The regulation of serum ascorbic acid levels is a coordinated process involving both intestinal absorption and renal excretion. At low concentrations, there is an increase in absorption within the small intestinal epithelium[131]. Conversely, at high concentrations, the

downregulation of the SVCT1 transporter limits both intestinal absorption and renal reabsorption[132]. In healthy individuals, the normal serum concentration of vitamin C ranges from 60 to 100 mmol/L[133, 134], with a physiological upper limit of approximately 200 mmol/L. Additionally, the SVCT2 transporter in platelets facilitates intracellular accumulation of ascorbic acid(Fig.2)[133, 135].

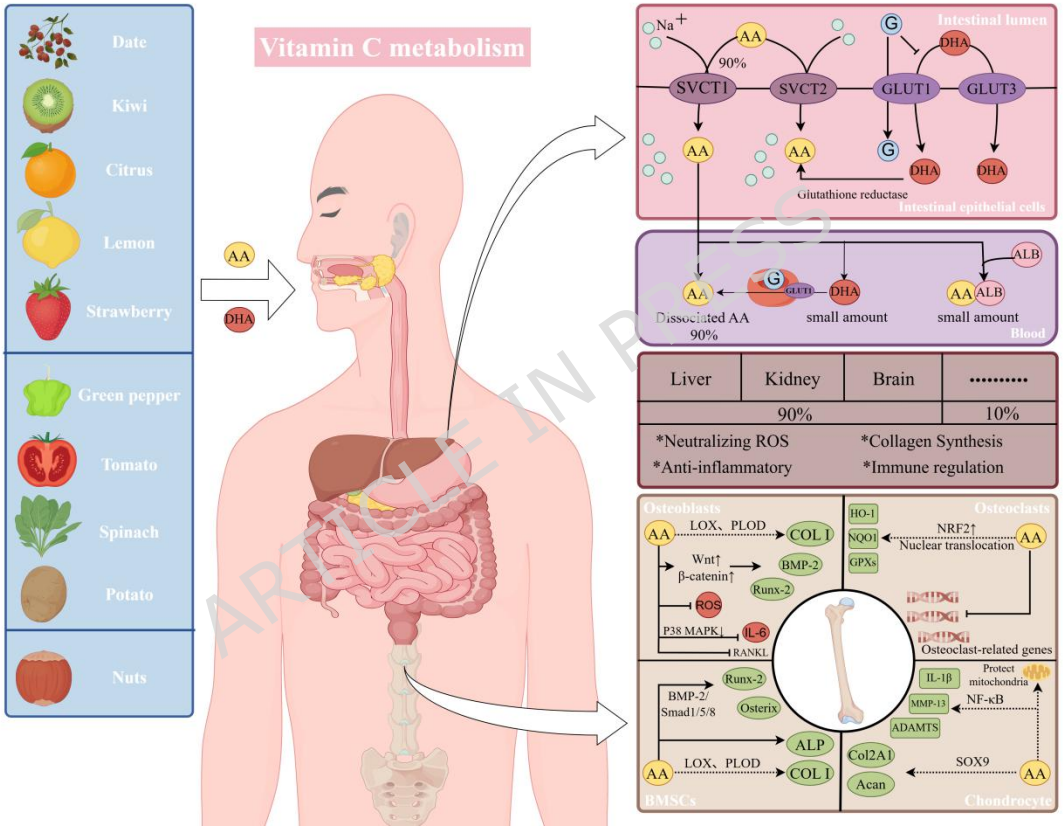

Figure.2 :Vitamin C Metabolism and Its Regulatory Effects on Bone

Previous research has established an independent correlation between vitamin C intake and bone mineral density in both premenopausal and postmenopausal women[136]. Additionally, vitamin C supplementation has been demonstrated to lower fracture risk in postmenopausal women[137].

This relationship may be attributed to the reduction in calcium levels associated with vitamin C deficiency[138, 139]. Recent studies suggest that the concurrent administration of vitamin C and vitamin D mitigates the risk of vertebral fractures and prevents bone density loss resulting from deficiencies in both vitamins[140]. Regarding bone health, the simultaneous deficiency of vitamin C and vitamin D exerts a more pronounced detrimental effect than the deficiency of either vitamin alone[140].Research has demonstrated that ascorbic acid possesses analgesic properties in the context of post-surgical pain following joint replacement[141]. In the treatment of fractures, the adjunctive administration of ascorbic acid has been shown to decrease the incidence of complex regional pain syndrome type I (CRPS-I)[142, 143]. Ascorbic acid, a potent scavenger of reactive oxygen species (ROS), may mediate its analgesic effects in rat models through this mechanism[144]. Furthermore, an imbalance in oxidative-reductive processes, induced by excessive ROS, is acknowledged as a critical molecular factor that impedes fracture healing[145]. Given its role as a prototypical ROS scavenger, ascorbic acid presents considerable potential in enhancing fracture healing. The subsequent sections will explore the molecular mechanisms by which ascorbic acid influences fracture healing and skeletal health, investigate its underlying pathways, and discuss its prospective applications in the field of fracture healing.

Early investigations suggest that vitamin C is integral to osteoclast differentiation through the stimulation of RANKL expression[146, 147].

Notably, mice deficient in vitamin C exhibit reduced levels of RANKL expression[148]. Simultaneously, evidence indicates that ascorbic acid (AA) decreases osteoclast survival rates[128]. In the context of osteoblasts, vitamin C administration has been shown to enhance cell viability, proliferation, and differentiation[128, 149, 150]. This effect has been corroborated in vitro, where AA facilitates osteoblast differentiation and activates transcription factors, thereby modulating the expression of genes associated with the bone matrix[150]. Furthermore, prior research involving ovariectomized Wistar rats has demonstrated that vitamin C fosters osteoblast proliferation, differentiation, and the expression of genes related to bone metabolism (such as BMP-2, SMAD1/5/8, GLI2, OCN, RANK, RANKL, TRAP, CTSK) via the Wnt/β-Catenin/ATF4 signaling pathway[151].In the context of addressing tibial bone defects, the synergistic application of ascorbic acid (AA) and growth factors has been shown to significantly enhance therapeutic efficacy[150]. Regarding chondrocytes, intra-articular administration of ascorbic acid has demonstrated substantial reparative effects on cartilage damage[152]. Nevertheless, AA has been observed to downregulate the expression of VEGF and TGF. While this modulation favors the regeneration of hyaline cartilage, it adversely affects fibrocartilage formation[153, 154]. Given that the fracture callus predominantly consists of fibrocartilaginous rather than articular hyaline cartilage, it is imperative to evaluate the overall impact of AA on this process. Bone marrow-derived mesenchymal stem cells, which are crucial

for maintaining skeletal health and facilitating fracture healing, are also influenced by vitamin A and its derivatives. Animal studies have revealed that AA enhances type I collagen secretion and increases ALP activity[155].Subsequent validation in human cells corroborated these findings, demonstrating increased calcium deposition and upregulation of osteogenic-related genes, including BMP-2, RUNX2, and COL1A2[156, 157]. Ascorbic acid 2-glucosides (AA 2Gs) are glycosylated derivatives of ascorbic acid, functioning as stable forms of Vitamin C[158]. In vitro experiments revealed that AA 2G-treated bone marrow-derived mesenchymal stem cells (BMSCs) exhibited enhanced proliferation, facilitated angiogenesis in vascular endothelial cells, and promoted migration and collagen synthesis in NIH-3T3 cells. In vivo studies indicated that animals treated with AA 2Gc experienced accelerated wound healing and increased neovascularization. These effects may be attributed to AA 2G's regulation of TET2, which promotes BMSC demethylation, and activation of the PI3K/AKT pathway, leading to upregulation of VEGF expression[159].

4.3 Vitamin E

Vitamin E, particularly in the form of α-tocopherol, has emerged as a prominent subject of research due to its role as a fat-soluble antioxidant and its potential impact on the molecular mechanisms and signaling pathways influencing osteocytes. Recent interdisciplinary studies have demonstrated that vitamin E intake is positively associated with bone mineral density

(BMD) in elderly populations, with higher intake levels correlating with a significant reduction in fracture risk[160, 161]. Data from the U.S. National Health and Nutrition Examination Survey (NHANES) further support these findings, revealing a significant association between increased vitamin E consumption and a decreased risk of osteoporosis, particularly among individuals with a history of fractures, thereby establishing a linear relationship with bone health[160]. Epidemiological evidence suggests that low levels of vitamin E are linked to reduced bone mineral density (BMD), especially in postmenopausal women, where insufficient vitamin E is considered a potential risk factor for osteoporosis(Fig.3)[160, 162].

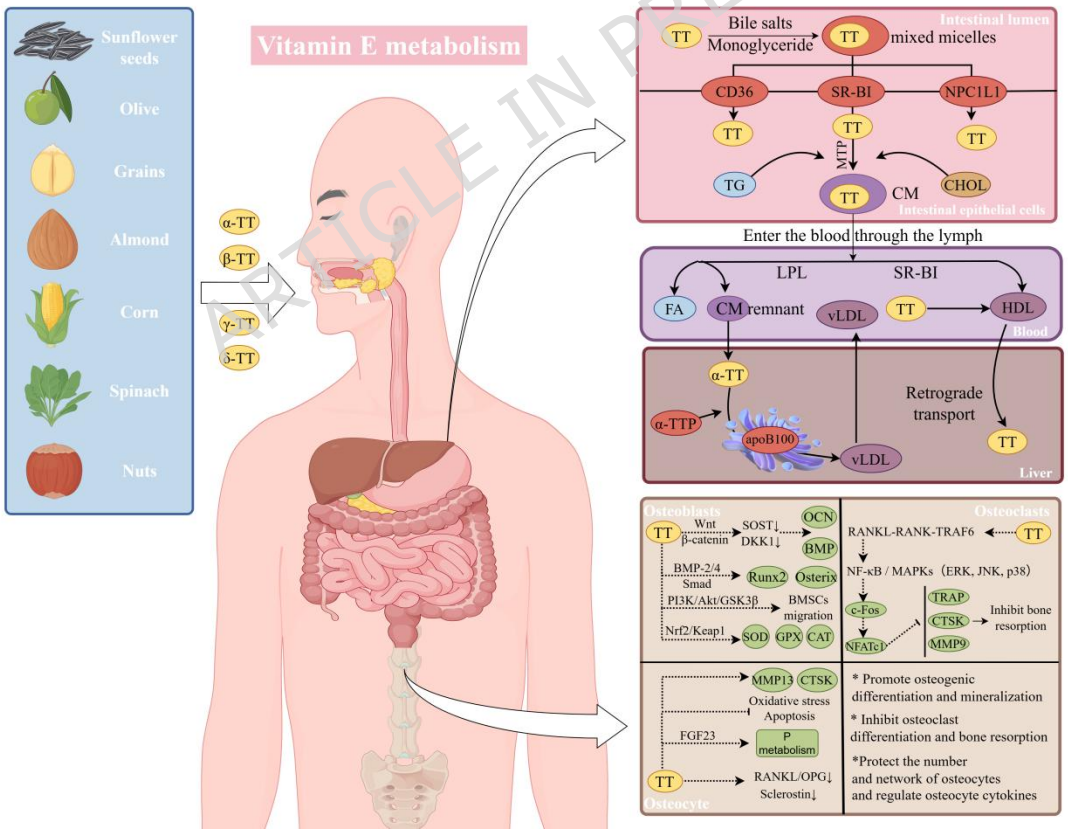

Figure.3:Vitamin E Metabolism and Its Regulatory Effects on Bone

The antioxidant properties of vitamin E constitute a crucial physiological mechanism for maintaining bone health. Oxidative stress within bone tissue significantly contributes to the development of osteoporosis and impedes fracture healing[163]. By neutralizing free radicals and reactive oxygen species (ROS), vitamin E reduces oxidative damage to bone cells, thereby safeguarding bone integrity[161, 164]. Research suggests that vitamin E can attenuate oxidative stress caused by external factors such as alcohol consumption and smoking, leading to improvements in bone mineral density (BMD) and bone strength[161, 165]. Specifically, δ-tocotrienol (δ-TT) enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby reducing oxidative stress and preventing mitochondrial damage[166]. The Wnt/β-catenin signaling pathway is a pivotal mechanism through which vitamin E and its derivatives exert their regulatory effects. Vitamin E increases Wnt3a expression and activates the β-catenin signaling pathway, which in turn promotes osteoblast proliferation and differentiation, ultimately stimulating bone formation[167-170].δ-Tocotrienol (δ-TT) facilitates the migration and differentiation of MC3T3-E1 and bone marrow stromal cells (BMSC), with partial dependence on the Akt pathway and a synergistic interaction with the Wnt signaling pathway[171]. The RANKL/RANK/OPG signaling axis is pivotal in regulating bone resorption. Vitamin E has been shown to inhibit osteoclastogenesis and decelerate bone resorption by downregulating RANKL expression and enhancing OPG levels[172]. Research

demonstrates that α- and γ-tocotrienols (α-/γ-TT) initially suppress the induction of c-Fos and NFATc1, leading to a reduction in the expression of tartrate-resistant acid phosphatase (TRAP/ACP5), cathepsin K (CTSK), matrix metallopeptidase 9 (MMP9), and dendritic cell-specific transmembrane protein (DC-STAMP/Tm7sf4), among other markers, thereby decreasing bone resorption activity in mature osteoclasts[173]. Additionally, γ-TT has been found to inhibit interleukin-17 (IL-17)-induced RANKL production and Th17 cell differentiation in rheumatoid arthritis contexts, consequently reducing osteoclastogenesis[174]. This mechanism contributes to the preservation of bone mass in conditions such as osteoporosis or excessive bone resorption and supports the recovery of bone density[175, 176]. Furthermore, the bone morphogenetic protein (BMP) signaling pathway is also integral to the process of bone formation.Vitamin E, particularly its derivatives such as γ-tocopherol and δ-tocopherol, has been shown to upregulate the expression of bone morphogenetic protein-2 (BMP-2) and promote osteogenic differentiation[167]. This effect is mediated through the BMP/Smad signaling pathway, which further enhances bone mineralization and osteocyte functionality[167, 176]. Specifically, vitamin E increases the expression of BMP-2 and BMP-4, subsequently driving the transcription factors RUNX2 and Osterix (SP7) via the Smad axis, thereby facilitating osteoblastic differentiation and mineralization[170, 177]. Additionally, vitamin E enhances the expression of key markers associated with bone formation, such as osteocalcin (OCN), ALP, LRP6, and COL1A1,

which collectively promote bone mineralization[177]. Furthermore, vitamin E significantly reduces levels of the bone resorption marker C-terminal telopeptide of type I collagen (CTX), thereby inhibiting bone resorption and supporting bone healing[175]. However, caution is warranted regarding high-dose vitamin E supplementation, as excessive intake may disrupt other aspects of bone metabolism, potentially leading to adverse effects[162]. Consequently, future research should prioritize the evaluation of optimal dosages and administration methods for vitamin E to maximize its clinical efficacy.

In summary, the antioxidant properties of vitamin E, when administered at suitable doses, substantially facilitate fracture healing. Empirical evidence suggests that vitamin E expedites this process by diminishing bone resorption and fostering new bone formation. In specific animal models, supplementation with vitamin E significantly accelerates fracture healing rates and enhances bone recovery following fractures[161, 172]. The dual capacity of vitamin E to stimulate bone formation and suppress bone resorption positions it as a promising adjunctive therapeutic agent for enhancing fracture healing[162, 167].

4.4 Vitamin K

Vitamin K, a representative fat-soluble vitamin, primarily comprises subtypes such as vitamin K1 (phylloquinone) and vitamin K2 (menaquinones, MK-n). Notably, MK-4 can be synthesized from K1 or K3 within tissues,

whereas MK-7 is derived from fermented foods and is characterized by a longer half-life and enhanced bioavailability, thereby rendering it more effective in fulfilling the requirements of extrahepatic tissues, such as bone and blood vessels. The bone-related regulatory roles of vitamin K can be broadly categorized into two pathways: the classical carboxylation pathway (GGCX/VKORC1 cycle), which imparts Ca^{2+}/hydroxyapatite-binding activity to osteocalcin (OCN) and matrix Gla protein (MGP), facilitating bone matrix mineralization and inhibiting abnormal calcification in soft tissues and chondroid areas[178]; and the regulation of Gla-rich protein, periostin, and protein S, which contribute to the homeostasis of the extracellular matrix[179].An alternative mechanism involves non-carboxylation, wherein vitamin K2 functions as a ligand for the steroid and xenobiotic receptor/pregnane X receptor (SXR/PXR)[180], leading to the upregulation of osteogenesis-associated genes, including CYP3A4, MSX2, Tenascin-C, BMP-2, and other genes related to osteogenesis and the extracellular matrix. In osteoblasts, specifically, vitamin K2 activates the PXR/SXR-Msx2 signaling axis and induces protein kinase A (PKA) phosphorylation, thereby promoting the transcriptional upregulation of collagen and matrix-associated genes, which facilitates osteogenic differentiation and mineralization. Mice deficient in PXR exhibit bone hypoplasia and increased bone resorption, underscoring the essential role of the vitamin K-PXR signaling pathway in maintaining bone homeostasis at the genetic level[180-182]. In inflammatory microenvironments, vitamin K2 inhibits

interleukin-1β (IL-1β)-induced proliferation of human osteoblasts (SaM-1) by downregulating osteocalcin (OC) carboxylation. This inhibitory effect is synergistic with vitamin K2's suppression of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, thereby contributing to the restoration of normal differentiation programs.MK-4 has been shown to specifically induce the expression of GDF15 and STC2 through a PKA-dependent pathway[182, 183]. Simultaneously, vitamin K inhibits the activation of NF-κB, reduces RANKL levels, and upregulates OPG, thereby suppressing osteoclastogenesis. Additionally, it positively influences redox homeostasis and mitochondrial ATP production, with MK-7 exhibiting particularly superior efficacy. Epidemiological studies suggest that a diet rich in MK-7 is associated with a reduced risk of hip fractures and an increase in bone mineral density. Furthermore, research indicates that diets high in MK-7 can mitigate bone loss in postmenopausal women[184]. In specific populations experiencing functional vitamin K deficiency, such as dialysis patients with renal failure, K2 supplementation has been found to improve VKDP carboxylation and bone markers(Fig.4)[185].

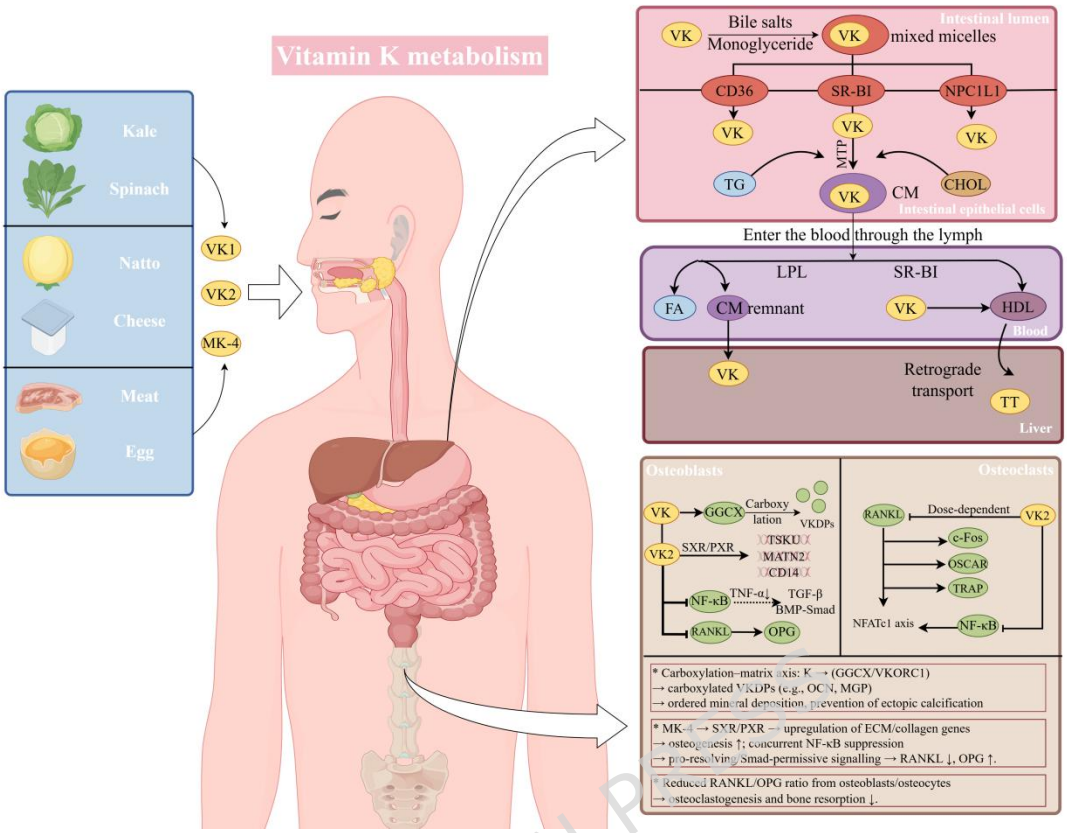

Figure.4:Vitamin K Metabolism and Its Regulatory Effects on Bone

In cellular biology, vitamin K, particularly K2, is crucial for bone cell functions. It aids mesenchymal stem cells in becoming bone cells by increasing markers like RUNX2, ALP, OCN, and BMP-2, and supports nodule formation[186]. Additionally, VK2 inhibits apoptosis mediated by Fas/Bax and induces autophagy through the activation of LC3 and Beclin1[187]. It further facilitates the transformation of osteoblasts into osteocytes. An area of particular interest, meriting further exploration, is vitamin K's dual regulatory effect on bone matrix formation and osteogenesis via the SXR/PXR and PKA-GDF15/STC2 pathways[182]. Moreover, VK2 functions as a mitochondrial electron carrier, thereby

supporting ATP production and reducing reactive oxygen species (ROS) levels to maintain bone metabolic homeostasis[188]. In relation to osteoclasts, VK2 downregulates RANKL, upregulates OPG, inhibits NF-κB and TRAP-positive cell proliferation, and induces osteoclast apoptosis in certain models[182, 189].Recent research has demonstrated that MK-4[190] can directly induce apoptosis in osteoclasts[187]. In the context of osteocytes, vitamin K2 (VK2) has been shown to enhance osteocyte density and lacunar occupancy in a glucocorticoid/sciatic nerve transection model, indicating its protective role against cortical bone porosity and its positive impact on bone quality[182]. Regarding osteoblasts, vitamin K facilitates endosteal homeostasis and remodeling through the modulation of MGP/Periostin and SXR/PXR-mediated matrix gene clusters[178, 179]. Furthermore, there are mutual regulatory interactions between vitamins K and D[191]. The ratio of vitamin D3 to K3 has been found to affect the expression of genes involved in bone and calcium metabolism, such as ALP, TRAP, PTHRP, and STC2. Achieving an optimal balance between these vitamins is crucial for promoting bone formation and remodeling, whereas improper combinations may have adverse effects. A prospective trial on spinal fusion revealed that osteoporotic patients undergoing ELIF who received a combination of VK2 and VD3 achieved fusion rates of 86.1% at 3 months and 91.7% at 6 months, which were significantly higher than those observed in the control group. The biological responses included a short-term increase in P1NP and a slight decrease in β-CROSS, although no significant changes in bone

mineral density (BMD) were observed within the first 6 months[192].During fracture healing, vitamin K reduces inflammatory osteoclast activity by inhibiting NF-κB and aids in proper calcification through MGP carboxylation in the early stage. In the callus formation stage, the SXR/PXR-BMP-2/MSX2/TNC and MK-4-PKA-GDF15/STC2 pathways, along with OCN carboxylation, enhance matrix maturation and mineralization. In the remodeling phase, RANKL/OPG balance prevents excessive resorption, maintaining callus integrity.

4.5 Vitamin B

Vitamin B is integral to the regulation of fracture healing processes, functioning through two primary pathways. The first is the one-carbon metabolism–homocysteine (Hcy) axis, which is mediated by vitamins B2, B6, folate, and B12 and represents a significant pathway. The second is the NAD^+/deacetylase axis, mediated by niacin, nicotinamide, and their precursors, which also plays a vital role in bone metabolism regulation. Numerous cohort studies have demonstrated that elevated Hcy levels and low vitamin B6/B12 status are positively correlated with an increased risk of osteoporotic fractures[193-195]. Conversely, excessive supplementation with vitamins B6 and B12 has been associated with a heightened incidence of hip fractures[196]. These observations may be attributed to a "threshold effect" involving vitamin B2's role in one-carbon metabolism[197].From a molecular biology standpoint, the role of B vitamins in bone metabolism is intricately

linked to their regulatory effects on bone-related cellular processes. Studies have demonstrated that vitamins B2, B6, folate, and B12 influence osteogenesis-associated markers such as Runx2, Osx, and Dlx5 by stabilizing theS-adenosylmethionine/S-adenosylhomocysteine (SAM/SAH) ratio and modulating methylation through the one-carbon metabolism/Hcy axis[198]. Furthermore, Vitamin B12 has been shown to directly influence bone growth and activity via the taurine–growth hormone/insulin-like growth factor-1 (GH/IGF-1) pathway[199]. Although B vitamins facilitate homocysteine clearance[200], elevated levels of homocysteine can promote osteoclastogenesis by increasing reactive oxygen species (ROS) and RANKL, thereby impacting NF-κB/ERK signaling pathways[201]. Additionally, homocysteine inhibits lysyl oxidase (LOX) expression and activity[202], which is a key factor in the reduction of bone matrix strength and contributes to impaired fracture healing quality[203, 204].The clearance of Hcy by vitamin B6 is contingent upon the transsulfuration pathway, involving cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CGL)[205]. Furthermore, the vitamin B3 family, which includes niacin, nicotinamide, nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN), significantly influences bone-related cells through the regulation of the NAD^+/deacetylase axis. NAD^+ enhances osteogenesis by maintaining the activity of sirtuin 1 (SIRT1), which is associated with ALP, collagen type I alpha 1 (COL1A1), and osteocalcin (BGLAP/OCN)[206]. It also preserves mitochondrial homeostasis via the SIRT3-FOXO3A-PGC1α pathway and

exhibits anti-inflammatory and antioxidant properties[200, 206]. Studies have demonstrated that in mice with compromised NAD^+ synthesis, NMN supplementation facilitates the proliferation of early soft and hard callus stem cells, interacts with macrophage signaling, and expedites fracture healing[207]. In osteocytes, a deficiency in vitamin B may result in an imbalance between bone formation and resorption[57, 208, 209]. Additionally, research suggests that folate mitigates homocysteine-induced endoplasmic reticulum stress in osteoblasts via the PERK-ATF4-CHOP pathway[210].

Throughout the various stages of fracture healing, specific biochemical processes play critical roles. During the inflammatory phase, members of the B3 family increase NAD^+/SIRT1 levels, thereby enhancing communication between macrophages and callus stem cells and fostering a pro-repair phenotype. Notably, NMN significantly accelerates the initiation of healing in murine models[211]. In the primary callus formation stage, folate and vitamin B12 are crucial for supporting DNA synthesis and the expansion of osteoprogenitor cells, while vitamin B6 is essential for maintaining crosslinking substrates and transsulfuration pathways, thereby preventing Hcy accumulation and reducing early matrix defects[205]. Elevated homocysteine interferes with lysyl-oxidase–mediated collagen crosslinking by downregulating LOX expression and activity, thereby compromising matrix stiffness and mechanical competence. Managing Hcy levels and supplementing with vitamins B6, B12, B2, and folate facilitate the formation of high-quality crosslinking[204]. In the remodelling phase,

thiamine (vitamin B1) attenuates RANKL–RANK–NF-κB signalling in osteoclast precursors, thereby reducing osteoclastogenesis and oxidative stress. While B3-NAD^+-Sirtuins refine osteoclast metabolic programs, promoting a balance between bone formation and resorption and facilitating trabecular remodeling[201].

4.6 Vitamin D

Vitamin D, a vital fat-soluble vitamin, is integral to the maintenance of bone homeostasis and the facilitation of bone repair. Its functions extend beyond the regulation of calcium and phosphorus metabolism, exerting direct influence on critical processes such as osteoblast differentiation, osteoclast formation and activation, and endochondral ossification[212, 213]. Both early clinical investigations and subsequent observational studies have demonstrated an association between vitamin D deficiency and delayed fracture healing, as well as an increased risk of fractures[214, 215]. Within the human body, vitamin D is primarily synthesized endogenously following ultraviolet radiation exposure to the skin, supplemented by dietary intake of vitamin $D_2$and vitamin D_3. Its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), binds to the vitamin D receptor (VDR), thereby regulating extensive gene transcription in the intestines, kidneys, parathyroid glands, and bone tissue. This regulation is crucial for maintaining calcium and phosphorus homeostasis, as well as for processes such as bone formation, resorption, and remodeling[216, 217].Recent studies have demonstrated that,

in addition to the well-known 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], metabolites such as 24R,25-dihydroxyvitamin D$_3$ [24R,25(OH)$_2$D$_3$] are not merely inactive byproducts but may actively contribute to the regulation of fracture healing[218, 219]. The physiological basis of vitamin D's role in bone repair is determined by its in vivo dynamics. Dietary vitamin D is predominantly absorbed in the small intestine, where it depends on a bile and lipid-rich digestive milieu. Once incorporated into chylomicrons, it enters the circulatory system via the lymphatic pathway. In the bloodstream, the majority of vitamin D and its metabolites are transported in association with vitamin D-binding protein (DBP). Following glomerular filtration, 25-hydroxyvitamin D [25(OH)D] is reabsorbed in the proximal tubule through megalin/cubilin-mediated mechanisms, a crucial process for maintaining circulating concentrations of 25(OH)D and 1,25(OH)$_2$D[220, 221]. The enzyme CYP24A1 facilitates the 24-hydroxylation pathway, producing 24,25(OH)$_2$D and the terminal metabolite calcitroic acid; the latter serves as a major end product of vitamin D catabolism and is excreted via bile[219, 222-224].

Vitamin D plays a crucial role in fracture healing by ensuring an adequate supply of calcium and phosphorus necessary for callus mineralization. The process of fracture repair, particularly the formation and mineralization of the cortical callus, imposes significant demands on calcium, phosphorus, and the local mineralization environment. The active form of vitamin D, 1,25(OH)$_2$D, enhances the absorption of calcium and

phosphorus by upregulating molecules involved in intestinal calcium transport. Alongside parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), it constitutes a regulatory axis for mineral homeostasis that facilitates callus maturation. A deficiency in vitamin D can result in hypocalcemia or a predisposition to secondary hyperparathyroidism, which in turn increases bone resorption and compromises the quality of new bone mineralization[225-228].The role of vitamin D in fracture healing extends beyond merely supplying the necessary components for mineralization. Emerging research indicates that vitamin D functions as a regulatory factor throughout the entire fracture healing process. This includes the initiation of inflammation, the formation of the cartilaginous callus, the mineralization of the bony callus, and the subsequent remodeling phase. Evidence from animal studies and basic research suggests that vitamin D facilitates repair by enhancing local blood circulation at the fracture site, promoting the proliferation and differentiation of osteoblasts, and augmenting mineralization[225].

The regulatory mechanisms of vitamin D exhibit variability across the distinct stages of fracture healing. In the initial inflammatory phase, vitamin D primarily modulates the fracture microenvironment [229, 230]. Post-fracture, the repair process is initiated by the formation of a local hematoma, infiltration of inflammatory cells, and the release of cytokines; however, excessive inflammation may impede callus formation[229, 231, 232]. Vitamin D exerts immunomodulatory effects that impact macrophages, inflammatory

mediators, and the local bone regenerative milieu, thereby facilitating a seamless progression through the inflammatory phase. As the process advances to the callus formation phase, vitamin D contributes to the maintenance of endochondral ossification by regulating chondrocyte maturation, hypertrophic differentiation, and the transformation of the matrix into mineralized bone tissue[233, 234].The formation and remodeling of the bony callus are intricately regulated by molecular mechanisms, particularly through the classical nuclear receptor pathway of vitamin D, which involves the $1,25(OH)_2D$–VDR–RXR axis[216, 235]. Upon entry into target cells, $1,25(OH)_2D$ binds to the vitamin D receptor (VDR), subsequently forming a heterodimer with the retinoid X receptor (RXR). This complex recognizes the vitamin D response element (VDRE), thereby modulating the transcription of a wide array of genes implicated in bone formation, mineral metabolism, and bone resorption. These genes include ALP, COL1A1, BGLAP/OCN, RANKL, OPG, as well as various molecules involved in calcium and phosphorus transport[213]. Furthermore, vitamin D is known to exert rapid non-genomic effects. Empirical studies have demonstrated that $1,25(OH)_2D$ can swiftly activate signaling pathways such as PI3K/Akt through VDR-dependent membrane-proximal mechanisms, thereby promoting osteoblast survival, inhibiting apoptosis, and diminishing caspase activity[236].

The regulation of RUNX2 by vitamin D is pivotal for osteogenesis[213, 237]. Vitamin D signaling enhances RUNX2 activity and its downstream

osteogenic pathways at specific differentiation stages, thereby augmenting the expression of osteogenic markers such as alkaline phosphatase (ALP), osteocalcin, and collagen, and facilitating the maturation and mineralization of the bone matrix[213, 238, 239]. It is important to acknowledge that the effects of vitamin D on osteogenic differentiation are contingent upon the stage of differentiation, cell maturity, and dosage. While low doses of vitamin D can stimulate precursor cell proliferation and early RUNX2 activation, higher doses may impede late-stage mineralization[239, 240]. Additionally, vitamin D interacts with the two principal osteogenic signaling pathways: BMP/Smad and Wnt/β-catenin[241].The BMP-2/Smad1/5/8 and Wnt/β-catenin signaling pathways collaboratively regulate RUNX2 activation and the initiation of the osteogenic program. Vitamin D, through the vitamin D receptor (VDR), plays a role in the integrated regulation of these pathways, thereby influencing the osteogenic differentiation of mesenchymal stem cells (MSCs) and bone matrix deposition[242, 243]. Vitamin D is crucial in bone remodeling, primarily via the RANKL/RANK/OPG pathway. Specifically, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] enhances the expression of RANKL in osteoblasts and osteocytes, facilitating the differentiation of osteoclast precursors[228, 244]. Simultaneously, it modulates osteoprotegerin (OPG) levels, thereby determining the overall intensity of osteoclastogenic signaling. In the context of endochondral ossification and chondrocyte regulation, vitamin D signaling interacts with factors such as parathyroid hormone-related protein (PTHrP), Indian hedgehog (Ihh), and fibroblast

growth factors (FGFs), potentially influencing vascular endothelial growth factor (VEGF)-mediated angiogenesis and matrix metalloproteinase (MMP)-related matrix remodeling, thus promoting the advancement of endochondral ossification[245, 246].

5 Interactions among vitamins during fracture healing and their impact on callus maturation

The preceding analysis of the impact of various vitamins on fracture healing and their involvement in bone metabolism highlights the pivotal importance of vitamin metabolism in fracture treatment and its potential clinical applications. However, vitamins do not function merely as isolated upstream regulators, the interactions and crosstalk among different vitamins are particularly significant. For instance, Vitamin D enhances the expression of matrix proteins such as osteocalcin (OCN) via the vitamin D receptor (VDR), while Vitamin K facilitates the formation of calcium-binding sites on OCN, matrix Gla protein (MGP), and growth arrest-specific protein 6 (GRP) through ɣ-carboxylation, thereby promoting mineralization. Studies suggest that the concurrent administration of vitamins D and K leads to more stable callus transformation and mineralization[180, 247, 248]. Furthermore, research indicates that the combination of vitamins E and C exhibits enhanced efficacy in antioxidant protection. Vitamin C aids in the regeneration of vitamin E in the aqueous phase, and their synergistic effect more effectively mitigates reactive oxygen species (ROS) damage to

osteoblasts, thereby safeguarding collagen and the extracellular matrix (ECM)[249-251].In addition to their synergistic roles in fracture healing, certain vitamins demonstrate antagonistic interactions that can reduce the effectiveness and utilization of beneficial vitamins. Research has shown that excessive intake of vitamin A can impair the calcium-promoting effects of vitamin D through various mechanisms and expedite bone resorption[97, 107]. Epidemiological studies further suggest that high vitamin A to vitamin D ratios are associated with an increased risk of fractures[107]. Moreover, high doses of α-tocopherol have been found to inhibit vitamin K activity, primarily evidenced by elevated levels of undercarboxylated osteocalcin (ucOC) and an increased risk of bleeding[252].The inhibition of bone vitamin K-dependent proteins (osteocalcin/matrix Gla-protein) by vitamin K antagonists facilitates processes related to ectopic calcification[253]. These studies not only elucidate the interactions between vitamins and their role in fracture healing but also highlight the critical importance of meticulously regulating vitamin dosages in clinical settings. Despite extensive prior research on the interactions among vitamins and their derivatives, our understanding remains incomplete. Future research should focus on elucidating the specific mechanisms underlying individual variability, temporal differences, and spatial variations in vitamin dosage utilization. Such insights are invaluable for the monitoring, supplementation, and provision of adjunctive vitamin therapy in clinical fracture patients.

6 Discussion

This review synthesizes the impact of vitamins A, C, E, K, and B on fracture healing. The findings underscore the indispensable role of vitamins in fracture healing, bone metabolism, and overall bone health. Current research not only highlights the increased risk of osteoporosis and fractures, prolonged healing times, and diminished bone strength associated with vitamin deficiencies but also elucidates the interactions among vitamins during the fracture healing process. While the administration of relevant vitamins during fracture healing is undoubtedly advantageous, several critical considerations must be addressed. Firstly, the dosage and duration of vitamin exposure have dual effects on outcomes. For example, moderate levels of vitamin A facilitate osteogenesis; however, high-dose, long-term vitamin A administration increases the RANKL/OPG ratio and SOST/FGF-23 levels, resulting in enhanced bone resorption and abnormal mineralization.The effects of vitamins on fracture healing are highly context-dependent, exhibiting tissue and site specificity. During the initial inflammatory phase, the emphasis should be on modulating inflammation and safeguarding vascular regeneration. In this context, the antioxidant properties of vitamins C and E, together with the anti-inflammatory and calcification-regulating functions of vitamin K, are crucial. In the subsequent phases of collagen formation and early mineralization, vitamin K assumes a central role through ɣ-carboxylation and the PXR-BMP-2/Msx2 axis, while B vitamins contribute to the maintenance of DNA synthesis and

methylation homeostasis. During the remodelling phase, vitamin K contributes to restraining excessive bone resorption by shifting the RANKL/OPG balance, whereas B-vitamin–dependent NAD^+–sirtuin signalling in osteoblasts, osteoclasts and osteocytes fine-tunes cellular energy metabolism and promotes coupled bone formation and resorption.In addition, vitamins engage in complex synergistic and antagonistic interactions: Vitamin D enhances osteocalcin (OCN) expression through the vitamin D receptor (VDR), while Vitamin K facilitates mineralization via carboxylation; their combined application further supports callus maturation. Vitamin C regenerates oxidised vitamin E in the aqueous phase, and together they scavenge reactive oxygen species in both aqueous and lipid compartments, thereby protecting the extracellular matrix and collagen, the extracellular matrix (ECM) and collagen. Conversely, excessive Vitamin A intake may diminish the calcium-promoting effects of Vitamin D, and α-tocopherol could disrupt the cycling of Vitamin K. Moreover, Vitamin K antagonist (VKA) therapy impedes the carboxylation of bone vitamin K-dependent proteins (VKDP) and disrupts mineralization homeostasis, highlighting the necessity for personalized clinical management. In conclusion, the application of vitamins in fracture healing transcends mere additive supplementation and necessitates scientifically tailored, individualized vitamin therapy. It is imperative to first ascertain the appropriate type, dosage, and duration of vitamin exposure, taking into account individual differences, nutritional status, fracture duration, and the

specific stage of healing prior to administration.Concurrently, vitamins should be combined according to specific conditions, such as the pairing of vitamin D with vitamin K or vitamin C with vitamin E. a demand-driven strategy using the minimum effective dose appears advisable. It is important to note that for high-risk groups susceptible to imbalances in vitamin-nutrient and bone metabolism—such as individuals with renal insufficiency (indicative of functional vitamin K deficiency), those using glucocorticoids, individuals with a history of smoking or high alcohol consumption, the elderly or postmenopausal women, and those experiencing malnutrition—a closed-loop management approach integrating biomarker assessment, disease staging and individualised combination therapy should be adopted.During the course of treatment, it is imperative to monitor alterations in vitamins and their derivatives, along with factors involved in pertinent biochemical pathways, to facilitate the adjustment of dosages and combinations as necessary. For instance, the sufficiency of vitamin K and the homeostasis of methylation can be evaluated through the ucOC/total OC ratio, matrix Gla-protein (MGP), Hcy, and the S-adenosylmethionine/S-adenosylhomocysteine (SAM/SAH) ratios. In conclusion, the effective application of vitamins in the context of fracture healing is not predicated on the notion that "more is better," but rather on the principle of administering "the right amount at the right stage, in the right combination, targeting the appropriate pathway," with adjustments informed by quantifiable biomarkers.

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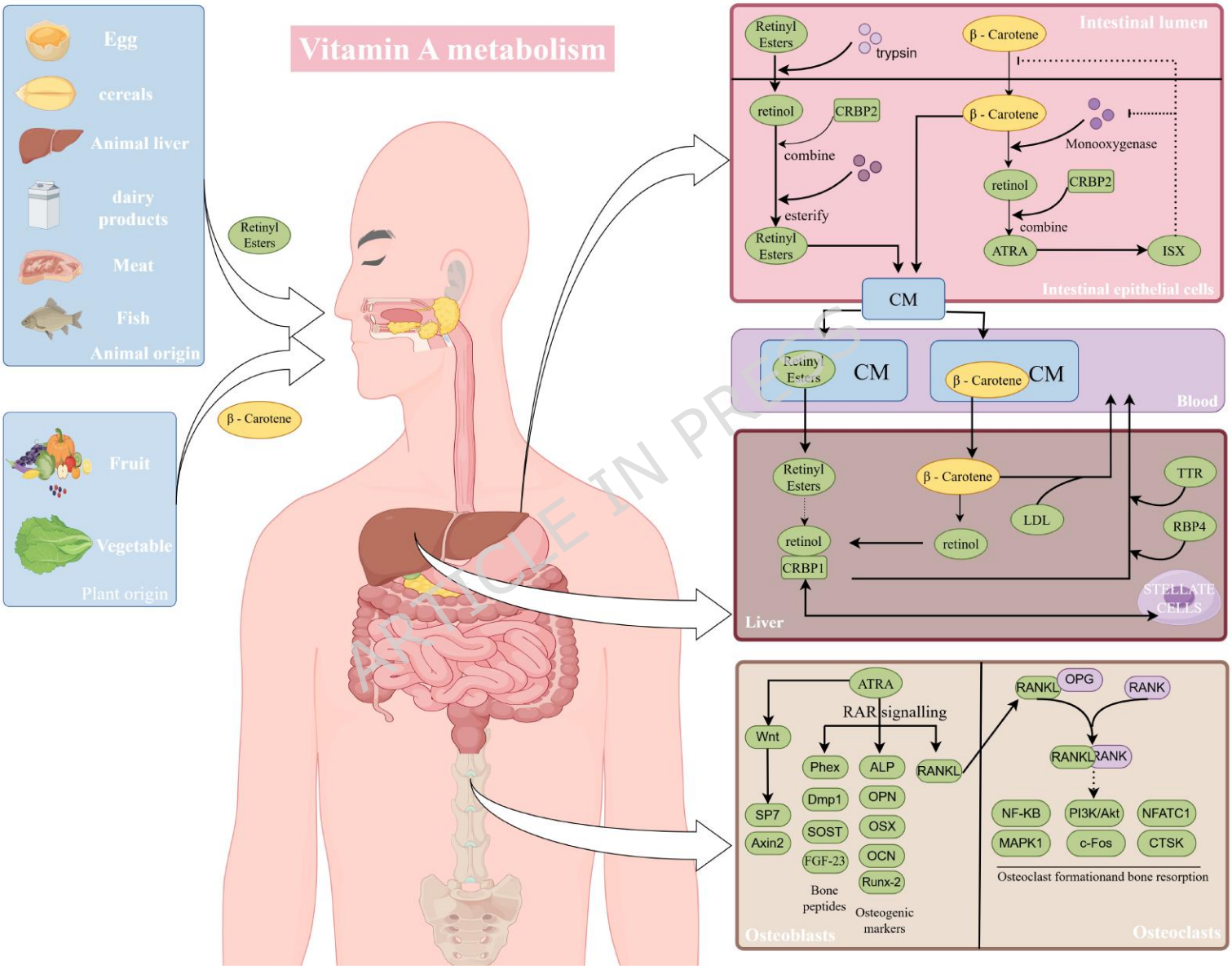

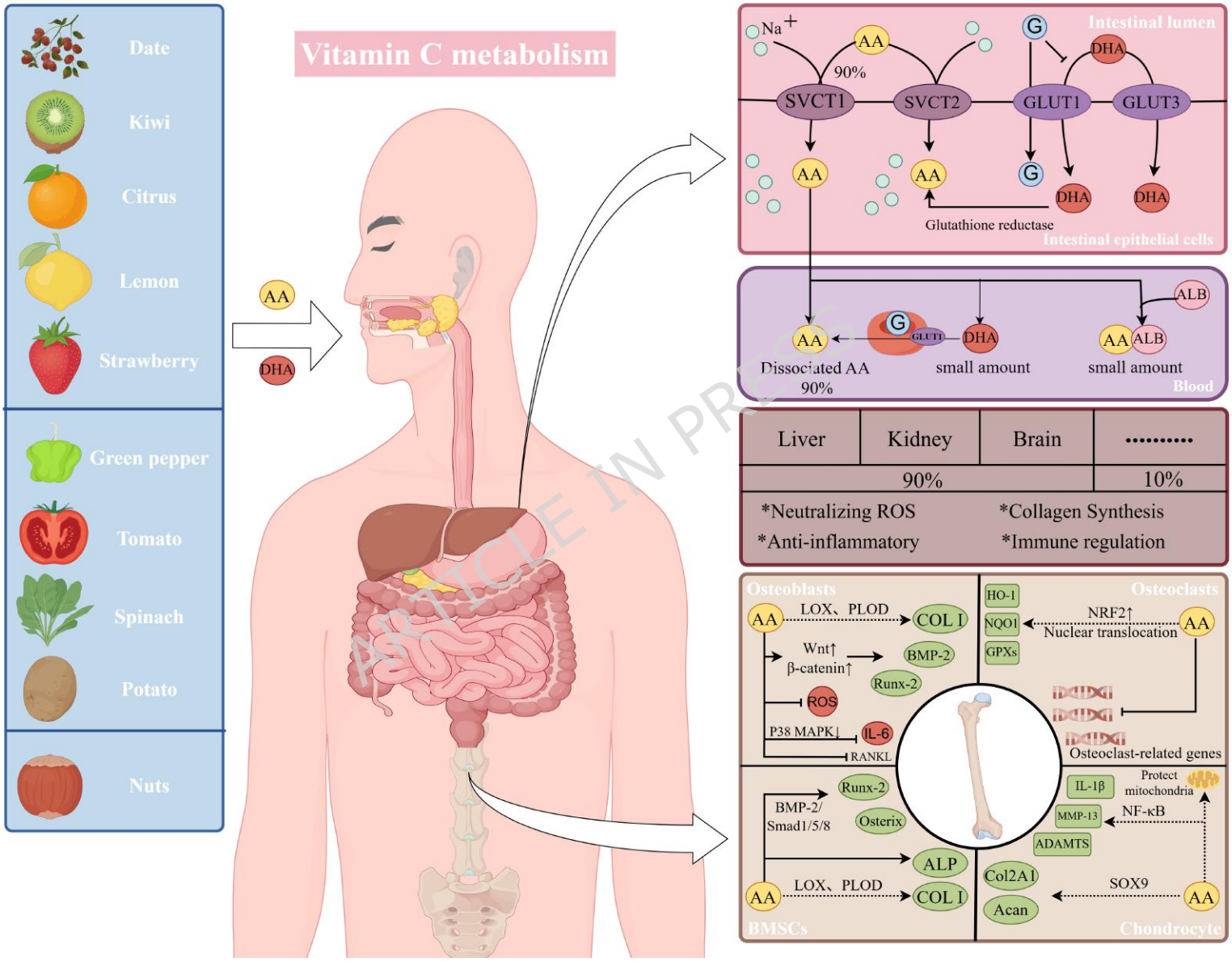
Vitamin C metabolism
Date
Kiwi
Citrus
Lemon
Strawberry
Green pepper
Tomato
Spinach
Potato
Nuts
AA
DHA
Na+
AA
90%
SVCT1
SVCT2
GLUT1
GLUT3
G
DHA
Intestinal lumen
Glutathione reductase
Intestinal epithelial cells
ALB
Dissociated AA
90%
small amount
small amount
Blood
Liver
Kidney
Brain
90%
10%
*Neutralizing ROS
*Anti-inflammatory
*Collagen Synthesis
*Immune regulation
Osteoblasts
Osteoclasts
LOX、PLOD
COL I
Wnt↑
β-catenin↑
BMP-2
Runx-2
ROS
P38 MAPK↓
IL-6
RANKL
HO-1
NQO1
GPXs
NRF2↑
Nuclear translocation
Osteoclast-related genes
Protect
mitochondria
IL-1β
MMP-13
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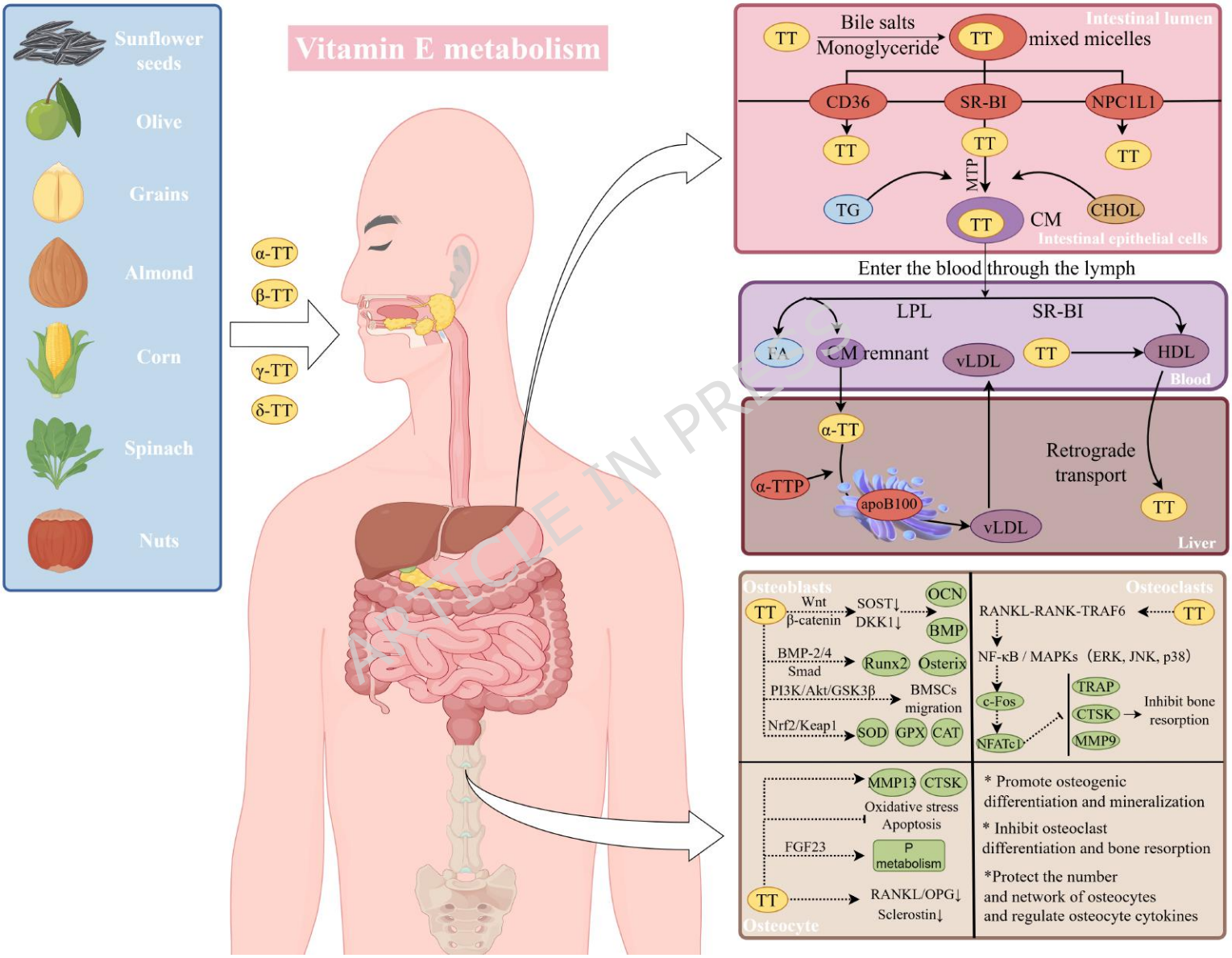

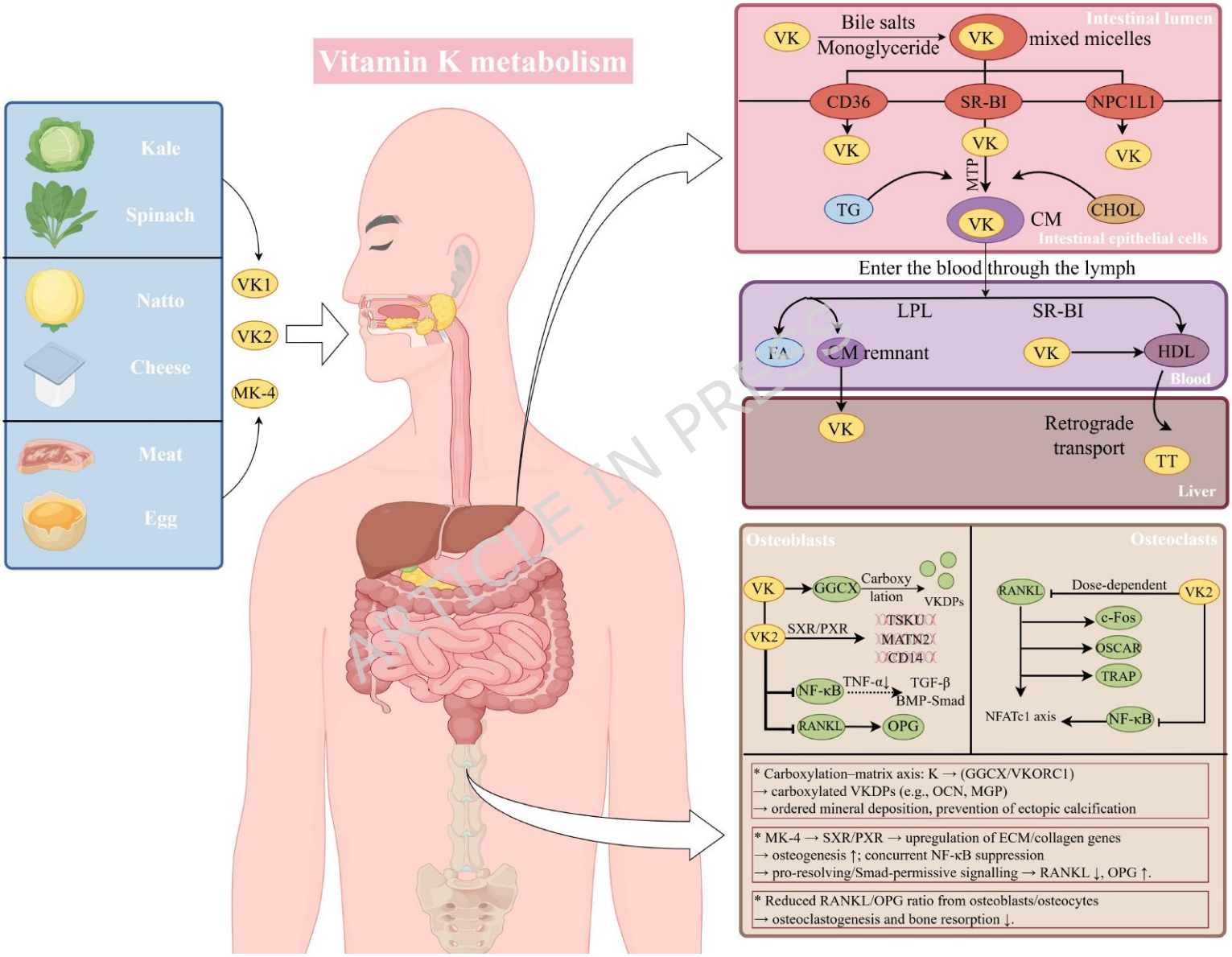

Table 1 | Key signalling pathways and downstream effectors involved in fracture healing at each stage

	Related pathways	Related factors	References
Inflammatory (hematoma) phase	*Thrombin- and factor Xa–mediated activation of PAR1/PAR2–RhoA/ROCK and ERK signalling *DAMP–TLR–NF-κB signalling driving TNF, IL-1 and IL-6 production *CCL2/CCR2 *Complement C5a–C5aR1 inflammatory–angiogenic signalling axis *Hypoxia–HIF-1α signalling driving VEGF and angiopoietin (ANGPT) expression	*MPO *CitH3/NETs *iNOS *CD86 *CD206 *IL-1β *TNF-α *CCL2/MCP-1 *SDF-1/CXCL12 *CXCR4 *VEGFA	[1-5]
Reparative phase – soft (fibrocartilaginous) callus formation	*TGF-β/BMP–Smad signalling pathway *Indian hedgehog (Ihh)–parathyroid hormone-related protein (PTHrP) signalling axis *MMP-9/-13–mediated extracellular matrix degradation and VEGF release *Canonical Wnt/β-catenin signalling (with reduced DKK1 and SOST expression)	*SOX9 *COL2A1 *ACAN *COL10A1 *HIF-1α *Ihh–PTHrP *MMP9 *MMP13 *DKK1 *RUNX2	[6-10]
Hard (bony) callus formation	*SLIT3–ROBO1/ROBO2 signalling axis *DLL4–Notch signalling in type H vasculature *Piezo1-mediated mechanotransduction activating YAP/TAZ and β-catenin signalling *PI3K–Akt–mTOR signalling pathway *Calcitonin gene-related peptide (CGRP) and substance P (SP) neuropeptide signalling *miR-26a, miR-29b and miR-21 modulating Wnt/β-catenin, RUNX2 and SP7 (Osterix) signalling	*SP7/OSX *ALP *COL1A1 *BGLAP/OCN *SPP1/OPN *BMP2 *BMP7 *Smad *Wnt *β-catenin *Piezo1 *YAP/TAZ *Integrin–FAK signalling	[11-16]
Remodeling phase	*RANKL–RANK–OPG signalling pathway *Osteoclast–osteoblast coupling pathways *Piezo1-mediated mechanotransduction activating YAP/TAZ and β-catenin signalling	*TRAP (ACP5) *CTSK *NFATc1 *RANKL/OPG *SOST/DMP1 *MMP9 *MMP13	[17-20]

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Table 2 Vitamins and their subtypes that modulate metabolic pathways involved in fracture healing

Subtype	Core pathways	Osteogenic differentiation markers	Bone resorption markers	References
Vitamin A Retinoids: retinol, retinal, retinoic acid, retinyl palmitate, 3,4-didehydroretinol Provitamin A carotenoids: β-carotene, α-carotene, β-cryptoxanthin	RAR/RXR signaling pathway Wnt/β-catenin signaling pathway TNFSF11 (RANKL)–TNFRSF11A (RANK)–TNFRSF11B (OPG)/NF-κB–NFATC1 signaling axis TGF-β/BMP–Smad signaling pathway VDR–RXR signaling pathway PPARG (PPARγ)–RXR signaling pathway RBP4–STRA6 signaling pathway	ALPL (ALP) COL1A1 RUNX2 SP7 (OSX) BGLAP (OCN) IBSP (BSP) SPP1 (OPN) TNFRSF11B (OPG)	ACP5 (TRAP) CTSK MMP9 CA2 DCSTAMP NFATC1 TNFSF11 (RANKL)	[1-6]
Vitamin C L-ascorbic acid Dehydroascorbic acid	Collagen hydroxylation–extracellular matrix axis Epigenetic regulation axis Wnt/β-catenin–ATF4–MAPK axis ROS–Nrf2–NF-κB axis PHD–HIF–microenvironment axis Uptake and supply axis (SLC23 family)	RUNX2 SP7 (OSX) ALPL (ALP) COL1A1 COL1A2 IBSP (BSP) SPP1 (OPN) BGLAP (OCN)	NFATC1 FOS (c-FOS) ACP5 (TRAP) CTSK MMP9 CA2 ITGB3 DCSTAMP TNFRSF11A (RANK) TNFSF11 (RANKL)	[7-14]
Vitamin E α-, β-, γ-, and δ-tocopherol α-, β-, γ-, and δ-tocotrienol	Wnt/β-catenin osteoanabolic axis Nrf2–HO-1/NQO1 antioxidant axis TNFSF11 (RANKL)–TNFRSF11A (RANK)–NF-κB/MAPK–NFATC1 osteoclast-lineage axis	RUNX2 SP7 (OSX) ALPL (ALP) COL1A1 BGLAP (OCN) BMP2 IBSP (BSP) SOST DKK1	FOS (c-FOS) NFATC1 TNFSF11 (RANKL) ACP5 (TRAP) CTSK MMP9 ITGB3 ATP6V0D2	[15-20]

Subtype	Core pathways	Osteogenic differentiation markers	Bone resorption markers	References
	Mevalonate–small GTPase axis Inflammatory mediator axis α-tocopherol phosphate (α-TP)–p38α–MITF axis		DCSTAMP	
Vitamin K Vitamin K1 (phylloquinone) Vitamin K2 (menaquinones) Vitamin K3 (menadione)	GGCX–VKORC1 vitamin K cycle axis SXR (PXR) nuclear receptor axis Vitamin D–vitamin K synergistic axis TAM receptor–GAS6/PROS1 axis	RUNX2 ALPL (ALP) COL1A1 SP7 (OSX) IBSP (BSP) SPP1 (OPN) DMP1	ACP5 (TRAP) CTSK MMP9 DCSTAMP OCSTAMP (downregulated)	[21-25]
Vitamin B complex Vitamin B1 (thiamine) Vitamin B2 (riboflavin) Vitamin B3 (niacin) Vitamin B5 (pantothenic acid) Vitamin B6 (pyridoxine) Vitamin B7 (biotin) Vitamin B9 (folate) Vitamin B12 (cobalamin)	One-carbon metabolism–homocysteine (Hcy)–TNFSF11 (RANKL)/TNFRSF11B (OPG)–ROS–p38 MAPK axis NAD^+–sirtuin energy-sensing deacetylase axis (vitamin B3-responsive) AGEs–RAGE–Wnt/NF-κB axis	ALPL (ALP) RUNX2 SP7 (OSX) BGLAP (OCN) COL1A1 IBSP (BSP) SPP1 (OPN) P1NP AXIN2 LEF1	ACP5 (TRAP) CTSK MMP9 NFATC1 FOS (c-FOS) DCSTAMP ITGB3	[26-33]

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