

Carnosine and bone (Review)

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Abstract. Carnosine is a vital endogenous dipeptide that has anti-inflammatory, antiaging, anti-crosslinking, antitumor and immune regulatory effects. Numerous cell and animal model studies have proved that carnosine and its compounds promote the proliferation and differentiation of osteoblasts, inhibit osteoclasts and protect chondrocytes. They also regulate the cell cycle of bone progenitor cells and the differentiation of bone marrow mesenchymal stem cells, accelerate fracture healing, delay bone tumor development and ameliorate osteopenia induced by estrogen deficiency or disuse. The correlations between carnosine and activation signal molecules, pluripotent differentiation of bone marrow mesenchymal stem cells and interaction between bone cells are unclear. However, studies have proved that carnosine and its compounds have benefits in preventing and treating specific bone diseases. This makes them potential agents for the treatment of osteoporosis and bone tumors. The present review summarized the existing research on carnosine and its compounds in bone cells and tissue. It focused on the physiological function of carnosine and its compounds in the bone and their effect on bone metabolism-related diseases, thus providing support for developing new strategies for targeted therapy.

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1. Introduction

Bone homeostasis is a dynamic lifelong process involving all body systems. Aging, hypogonadism, inflammation, malnutrition and alcohol abuse are some of the known risk factors for osteoporosis or other bone-related diseases. These risk factors cause an imbalance of osteoclastic bone resorption and osteoblastic bone formation, leading to abnormal bone mass (1-3). A recent meta-analysis showed that the commonest bone metabolism-related disease is primary osteoporosis, which includes postmenopausal and senile osteoporosis (4).

Carnosine is a water-soluble, small-molecule dipeptide with anti-inflammatory, antioxidant, antiaging and antitumor functions (5). *In vitro* and *in vivo* studies suggest that carnosine could promote the synthesis of new bone proteins by osteoblasts, increase alkaline phosphatase activity, induce differentiation of pluripotent mesenchymal stem cells into osteoblasts and chondrocytes, not myoblasts, inhibit bone resorption activity by osteoclasts without reducing their numbers and enhance protein formation in fracture healing (6,7). Carnosine could also reduce the damage of deleterious factors on the bone microenvironment and maintain local and systemic bone metabolism (8).

Therefore, *in vivo* carnosine and carnosine supplements are indispensable for bone homeostasis. They may be an effective method to prevent and improve multifactorial bone metabolic disorders such as those caused by aging or oxidative stress. The present review focused on the physiological role of carnosine in bone metabolism and its potential therapeutic use in different bone-related diseases (9,10).

2. Carnosine and its compounds

Carnosine is a natural bioactive dipeptide abundant in skeletal muscles and the nervous system consisting of β -alanine and L-histidine, under the co-catalysis of carnosine synthase and ATP, which synthesize a variety of carnosine-related compounds such as homocarnosine and acetyl carnosine. Carnosine chelates zinc ions in a way that is not toxic to the human body, performing a positive effect on bone metabolism (11), and the bioactivity of zinc carnosine (ZnC) is stronger

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than that of zinc sulfate or other carnosine chelates such as N-acetyl- β -alanine-L-histidine, and its mobilization is reactive oxygen species (ROS)-mediated (12,13). In addition, due to the unique binding characteristics of metals and hydroxyapatite in bone tissue, the zinc-chelating carnosine is absorbed easily in the intestinal tract and carnosine enhances the bioavailability of zinc ions by raising its intracellular uptake in bone tissue, which stimulates bone cells and collagen synthesis (14,15). A number of experimental studies found that the alanine group in ZnC improved the activity, mineralization, enzyme activity and nucleic acid metabolism of osteoblasts, and the activity and concentration of alkaline phosphatase (ALP) significantly increased in a time-dependent manner in culture media containing zinc carnosine (11).

Accumulated evidence shows the anti-oxidative stress and anti-inflammation effects of carnosine. On the one hand, it can enhance the scavenging and phagocytic activity of M1 macrophage (9), increase the expression of TGF- β , activate nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway and scavenge ROS (16); On the other hand, carnosine reduces the oxidative stress reaction by suppressing the release of inflammatory cytokines IL-1 β and IL-6, TNF- α and inhibiting NF- κ B in inhibiting the expression of inflammatory mediators NO and inducible nitric oxide synthase (iNOS) in lipopolysaccharide-induced RAW264.7 macrophages (17). Carnosine combines with advanced glycation end products (AGEs) and 4-hydroxynonenal (4-HNE) to reduce the expression of lipid and protein peroxidation (18). In addition, carnosine can also promote wound healing and chelate heavy metal ions (Zn²⁺ and Cu²⁺), thus decreasing intracellular metal-induced toxicity (19), as well as reducing lactic acid accumulation and buffering the pH values of muscles, thus increasing muscle endurance and alleviating fatigue (20).

ZnC also known as polaprezinc, or PepZin GI, is a new zinc peptide and the first zinc-related drug approved in Japan (21). Current clinical evidence confirms that ZnC and carnosine can both prevent diseases characterized by oxidative stress and/or neurodegenerative changes, such as diabetes and its concurrent kidney and neuropathy, depression, cerebral ischemia, Alzheimer's disease (AD), peptic ulcer and *Helicobacter pylori*-related gastritis (22). Studies have also shown a significant therapeutic effect of the non-hydrolyzed form of L-carnosine on influenza virus-induced pneumonia via NO and cytokine regulation (3,23). In addition, carnosine can also be used as an over-the counter food supplement to improve muscle tolerance or as a major component of cosmetics to exert its antioxidant and anti-aging effects (24). Although a large number of preclinical studies have been conducted *in vivo* and ZnC has been authorized by the FDA (6), the safety and efficacy of carnosine in bone metabolism-related diseases needs further verification.

3. Cell-specific effects of carnosine compounds on bone

Osteoblasts. Carnosine and its compounds serve critical roles in bone metabolism and mineralization. First, a series of short-term MC3T3-E1 cell cultures showed that ZnC could markedly increase the expression of target genes such as runt-related protein 2 (Runx2) and osterix, promote the DNA synthesis of osteoblasts and simultaneously increase

the collagen and calcium content in the matrix based on the newly synthesized bone proteins (25). This indicates that ZnC might be involved in nuclear transcription and protein translation. After a long-term MC3T3-E1 cell culture, ALP, a biochemical marker of osteoblast differentiation, increased significantly and its expression and activity changed in a time- and dose-dependent manner, which indicates that ZnC contributes to the proliferation, differentiation of osteoblasts (26). Second, bone resorption stimulating factors such as parathyroid hormone (PTH), IL-1 and prostaglandin E2 (PGE2) were shown to be effective inhibitors of bone formation by osteoblasts (27). ZnC (10^{-6} - 10^{-4} M) did not completely inhibit the effect of these bone resorption stimulators, but they could block the reduction of acid phosphatase and alkaline phosphatase activities and prevent intracellular glucose depletion and lactic acid accumulation (28).

At the molecular level, carnosine and its compounds at the same dose (10^{-6} - 10^{-4} M), could rescue the transcription of osteoblasts by decreasing the inhibition of NF- κ B signaling pathway by ROS or by enhancing the phosphorylation motifs of cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) (29); carnosine increases the expression of osteoblast-specific transcription factor Runx2/core-binding factor α 1 (Cbfa1) through active TGF- β /bone morphogenetic proteins (BMPs) and insulin-like growth factor 1 (IGF-1)/protein kinase C (PKC)/mitogen-activated protein kinases (MAPK) transduction signaling pathway and upregulates the mRNA expression levels of target genes osterix, osteocalcin (OCN), osteoprotegerin (OPG), IGF-1 and TGF- β , finally promoting the proliferation and differentiation of osteoblasts (6,30). Autocrine of TGF- β could also stimulate the differentiation of early osteoblasts, promote the recruitment of osteocytes and the expression of their matrix protein, regulate the formation of osteoblast and bone remodeling in coordination with PTH and regulate coupling in bone formation and bone resorption (31).

In conclusion, study results support the idea that carnosine compounds could directly and specifically promote the proliferation and differentiation of osteoblasts *in vivo* and *in vitro*. Probably, the mechanism is partly mediated by increasing protein kinase and protein phosphatase in osteoblasts, stimulating newly synthesized bone proteins in osteoblasts, enhancing the activity of aminoacyl-tRNA synthetase in zinc-activated translation and partially stimulating bone formation and calcification (Fig. 1). However, there are relatively few clues about the early stages of osteoblast differentiation, which require further research.

Osteoclastogenesis. Osteoclasts are multinucleated cells originating in mature monocyte-/macrophage-lineage cells and needed for bone growth and remodeling, maintenance of the bone structure and bone calcium metabolism throughout their life cycle. A number of bone resorptive factors can stimulate bone marrow macrophages (BMMs) to differentiate into osteoclasts, concomitant with an evident increase in the number of osteoclast-like cells and the decrease of bone calcium content. Under pro-inflammatory conditions, the uptake of carnosine by macrophages is markedly increased (32). A dose of 20 mM carnosine can reduce the release of pro-inflammatory cytokines and exert its anti-oxidant effect by changing the balance and polarization state

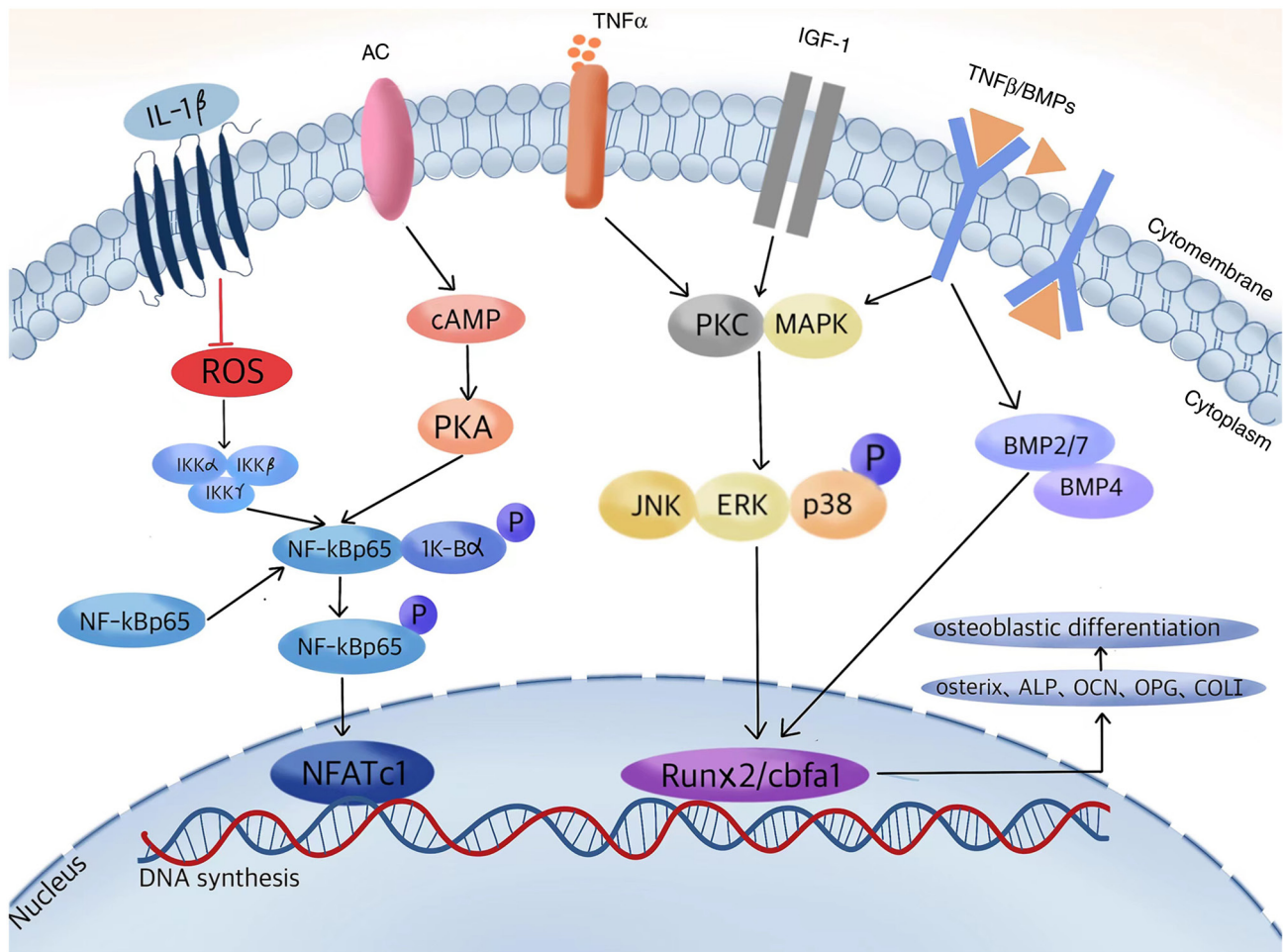


Figure 1. Proposed signaling transduction cascades of carnosine compounds in regulating osteoblastic differentiation. A series of carnosine-regulated related cytokines and molecules interact within osteoblasts to promote protein kinase phosphorylation and DNA synthesis, increasing the expression of downstream target genes and bone proteins, thereby stimulating bone formation. AC, adenylyl cyclases; IGF-1, insulin-like growth factor 1; BMP, bone morphogenetic protein; ROS, reactive oxygen species; NFATc1, nuclear factor of activated T cells 1; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; Runx2, runt-related protein 2; cbfa1, core-binding factor α 1; ALP, alkaline phosphatase; OCN, osteocalcin; OPG, osteoprotegerin; COL I, collagen I.

of macrophage M1/M2 (33). However, further studies are required to elucidate whether carnosine serves a protected role in abnormal bone loss by regulating the 'liquid' state of macrophage polarization.

Unlike osteoblasts, ZnC (10^{-6} - 10^{-4} M) directly inhibit the early stage of BMMs differentiation into osteoclast precursors, block the formation of pre-osteoclasts and its receptor activation of NK- κ B (RANK) expression and suppresses osteoclastogenesis without obviously inhibiting the function of osteoclasts (34). An experiment with mature osteoclasts showed that ZnC (10^{-5} M) could inhibit TNF- α production by osteoclasts and attenuate its stimulating effect on osteoclast differentiation. The results indicated that the inhibitory effect of ZnC on PTH-induced osteoclast cell formation was through PKC-mediated protein kinase activation (28,35). An *in vitro* study with cultured mouse marrow demonstrated that both zinc sulfate (10^{-6} M) and zinc-chelating dipeptide (10^{-6} M) could inhibit the TGF- β induced formation of tartrate-resistant acid phosphatase-positive cells, whereas these inhibitory effects were abrogated by egtazic acid (EGTA) (36). It further revealed that ZnC could inhibit the stimulatory effects of PTH on osteoclast-like multinucleated cell formation of mouse

marrow cells, through Ca $^{2+}$ -calcineurin-nuclear factor of activated T cells 1 (NFATc1) dependent activation of protein kinase C (PKC) (37).

By contrast, another *in vitro* study demonstrated that a dose of 50 μ M polaprezinc produced the opposite result, promoting BMMs and RAW264.7 cell differentiation into osteoclasts by enhancing the mRNA expression levels of NFATc1 and cathepsin K in osteoclasts as well as the transcriptional activity of yes-associate protein (38). However, this is in complete contrast to the effect of ZnC on osteoclasts confirmed in a previous study (34). The authors do not provide further statement on this point (38), so whether ZnC exerts dose- or condition-specific effects on osteoclastogenesis needs to be further studied.

Interaction between osteoblast-lineage cells and osteoclast-lineage cells. The interactions between osteoblasts and osteoclasts, as well as their precursors, serve a major role in determining the balance between bone formation and resorption. In addition to regulating the differentiation and function of osteoblast and osteoclast, as aforementioned, carnosine compounds are also reported to be involved in the interaction

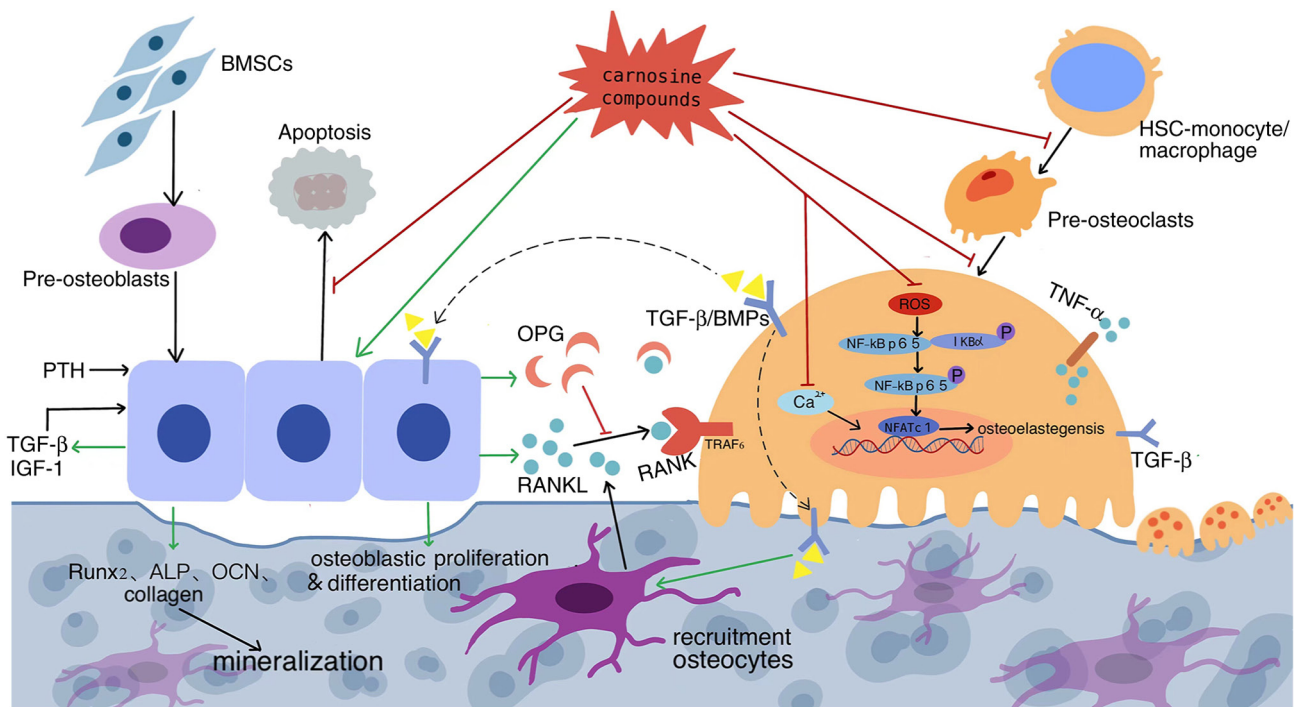


Figure 2. Dual regulation of carnosine compounds on cellular interactions between osteoblast lineage and osteoclast lineage cells. Carnosine stimulates osteoblast proliferation and differentiation, inhibits apoptosis, activates bone progenitor cell enrichment, whereas inhibiting BMM differentiation and pre-osteoclast migration to reduce osteoclastogenesis and through regulating the RANKL-RANK ratio, thus effectively inhibiting the formation, differentiation and apoptosis of osteoclasts and stabilizes bone mass. Its promoting effect is marked with green arrows and inhibiting effect with red. Dotted line indicate a proposed rather than confirmed effect. BMM, bone marrow macrophage; RANKL, receptor activator of NF- κ B ligand; RANK, receptor activation of NF- κ B; BMSCs, bone marrow mesenchymal stem cells; PTH, parathyroid hormone; IGF-1, insulin-like growth factor 1; OPG, osteoprotegerin; BMP, bone morphogenetic protein; HSCs, hematopoietic stem cells; NFATc1, nuclear factor of activated T cells 1.

between osteoblast-lineage cells and osteoclast-lineage cells, exhibiting beneficial effects on preserving bone mass.

An *in vitro* study confirmed that carnosine compounds could stimulate osteoblast-lineage cells obtained from the calvaria of weanling rats (3-week-old males) to secrete decoy receptor OPG and block the receptor activator of NF- κ B ligand (RANKL)-RANK interaction between osteoclast precursors and osteoclasts by upregulating the OPG/RANKL ratio (13). This effectively inhibits the formation, differentiation and apoptosis of osteoclasts and stabilizes bone mass. In addition, RANKL secreted by mature bone cells is involved in a series of cascade reactions of osteoclastogenesis and bone resorption (21). It has been clarified that when RANK is activated, it leads to phosphorylation of P65 and its upstream inhibitor protein I κ B, increased recruitment of TNF receptor associated factor 6 and nuclear translocation, subsequently promoting osteoclastogenesis (39). However, carnosine compounds negatively regulate the osteoclast process by inhibiting ROS production during this process (Fig. 2).

Consequently, under different physiological and pathological conditions, carnosine compounds at the dose of 10^{-6} - 10^{-4} M could promote bone marrow mesenchymal stem cells (BMSCs) to differentiate into osteoblasts, stimulate osteoblast activity and activate osteoprogenitor enrichment, inhibit BMMs differentiation into osteoclasts and pre-osteoclast migration, reduce osteoclast formation and directly or indirectly affect bone resorption of osteoclasts through cell communication between osteoblasts/stromal cells and osteoclasts progenitor cells. While the recently published study (38) drives debate on

their effects on osteoclastogenesis, more studies are needed to clarify this point.

Mesenchymal stem cells. Mesenchymal stem cells (MSCs) are a subset of cells derived from different tissues. BMSCs and periodontal ligament stem cells (PDLSCs) are involved in bone formation and regeneration. MSCs secrete cytokines through a paracrine and immune response. These cytokines are used in the treatment of a number of diseases (39).

Maeno *et al* (6) confirmed that carnosine compounds stimulate PDLSCs to produce more BMPs, including BMP-2, BMP-4 and BMP-7. This promotes the differentiation of MSCs into osteoblasts and/or chondrocytes. PDLSCs, derived from teeth, have been proven to differentiate into osteoblasts or fibroblasts. They maintain periodontal tissue by regulating bone formation and bone resorption and have stronger proliferative and anti-apoptotic abilities than BMSCs (40). Carnosine compounds also mediate the mesenchymal differentiation of PDLSCs into osteoblasts through an autocrine process. The expression of the osteogenic differentiation-related transcription factor RUNX2/Cbfa1 and the activity of ALP in PDLSCs were increased in a time-dependent manner with carnosine (6).

In vitro studies by Takada *et al* (41) showed that carnosine compounds could increase the expressions of Runx2/Cbfa1, transcription factor SOX9 and type X collagen (Col X), the marker of complete differentiation of chondrocytes) by mediating cartilage differentiation in C2C12 cells of mesenchymal myoblasts (42). By contrast, the expression of myogenic-related markers MyoD and desmin decreased significantly. This

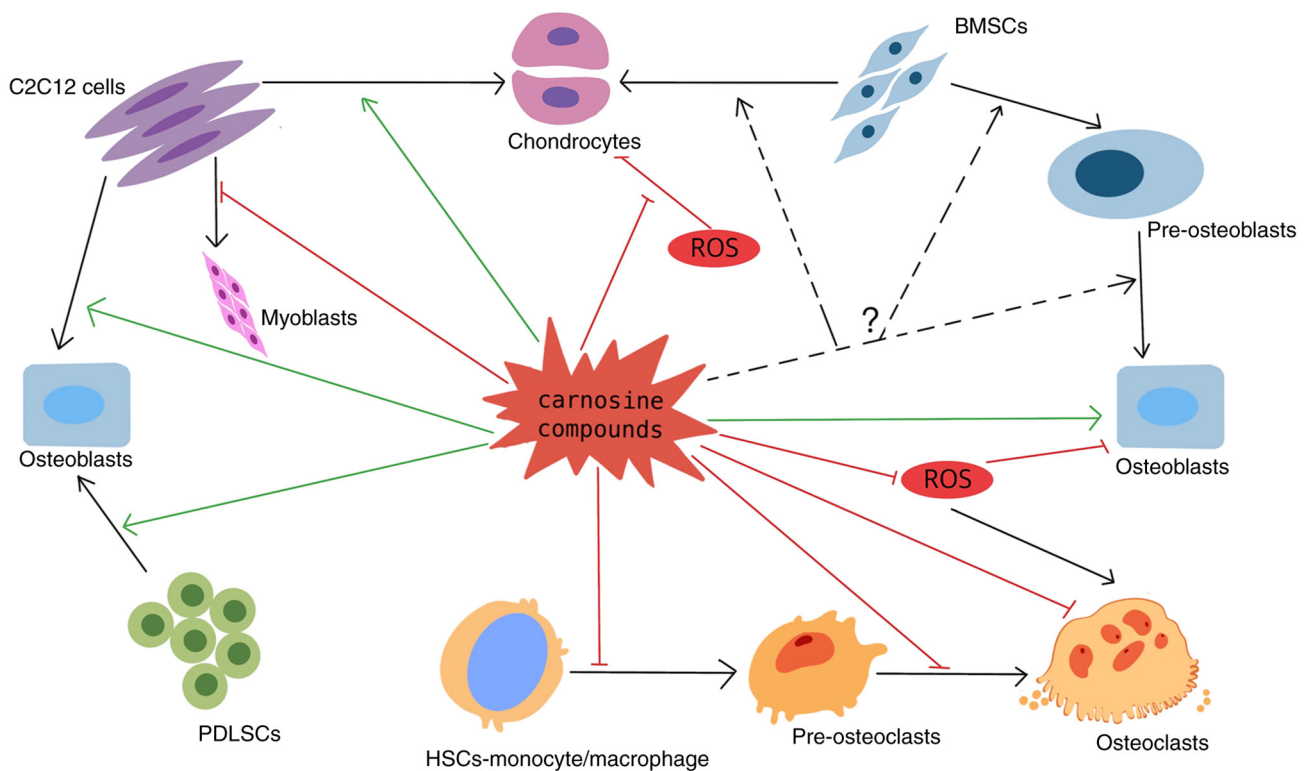


Figure 3. Cell-specific effects of carnosine compounds on selected cells participated in bone metabolism. Carnosine involves in regulating the differentiation of BMSCs, C2C12 myogenic stem cells, PDLSCs and BMs, as well as regulating the activity of chondrocytes, osteoblasts and osteoclasts by decreasing ROS. Its promoting effect is marked with green arrows and inhibiting effect with red. Dotted line indicate a proposed rather than confirmed effect. BMSCs, bone marrow mesenchymal stem cells; PDLSCs, periodontal ligament stem cells; ROS, reactive oxygen species.

indicates that carnosine induces C2C12 myogenic stem cells to differentiate into osteoblasts and/or chondrocytes and inhibits their transformation into myoblasts (Fig. 3).

4. Mechanism and the potential effect of carnosine compounds on bone-related diseases

Osteoporosis

Postmenopausal osteoporosis. Osteoporosis is the most common type of systemic degenerative bone disease. It emerges at all ages but it is predominantly diagnosed in postmenopausal women and older men. Postmenopausal osteoporosis is characterized by a progressive decrease in bone mineral density (BMD), deterioration of bone microarchitecture and an imbalance of bone formation and resorption regulated by osteoblasts and osteoclasts, respectively. This is accompanied by the accumulation of bone marrow fat resulting in compromised bone strength and an increased propensity for fragility fractures (43).

Postmenopausal osteoporosis may be based on the interaction of local cellular and molecular factors, including the deficiency of estrogen and the change of immune status of postmenopausal women. For example, T lymphocyte subtypes express TNF α , which increases the apoptosis of osteoblasts and indirectly stimulates the generation of osteoblasts through RANKL produced by B cells, as well as the changes of chronic inflammation phenotypes and cytokine expression (44). Excessive accumulation of AGEs and age-related obesity lead to an increased bone turnover

rate and decreased bone trabecula and cortical bone density, resulting in continuous bone destruction (31). Studies refer to 'the three-way regulation' model of bone metabolism, which consists of the imbalance of regulation of the coupling of bone vessels, osteoclasts and osteogenesis, which leads to a decrease in cortical thickness and an increase in cortical porosity (45,46). The histomorphology and cytokines related to bone metabolism in ovariectomized rats confirmed that the oral administration of ZnC (10-100 mg/kg/day) for 6 weeks can significantly increase zinc accumulation, bone protein synthesis and collagen contents in bone, completely block the bone loss of femoral trabecula and prevent the deterioration of cortical bone (47). Comparatively low doses of ZnC (10 and 30 mg/kg/day) possess the same ameliorating actions (48). Carnosine compounds have been proved to inhibit bone resorption in tissue culture, so reasonably, carnosine may partially inhibit osteoclast resorption. These results indicate that carnosine compounds have a direct stimulating effect on bone formation and calcification and effectively prevent BMD reduction in ovariectomized rats.

From the perspective of systemic nerve-bone axis regulation, the dysfunction of the autonomic nervous system (made up of the sympathetic and parasympathetic systems) is also frequent in elderly or postmenopausal osteoporosis patients (49). Some authors found that under the conditions of aging or menopause (estrogen deficiency), the imbalance of sympathetic and parasympathetic nerves in bones stimulates bone cells to overproduce neuropeptide Y, inhibits cAMP/PKA/CREB signaling pathway, weakens osteogenic

differentiation and enhances the adipogenic differentiation of BMSC, leading to bone-lipid imbalance and bone marrow obesity (49,50). Notably, carnosine is hypothesized to inhibit sympathetic nerve activity and promote parasympathetic nerve activity (51) and its effect may be to inhibit sympathetic nerve activity through the natural killer (NK) cells activity of spleen cells or regulate the autonomic nervous system through histidine H3 receptor (52,53). In brief, carnosine may provide new research avenues for postmenopausal and age-related bone metabolism through neuro-bone axis regulation.

Carnosine compounds are important because they could exert a broad-spectrum effect on the bone remodeling of osteoblasts and osteoclasts and inhibit ovariectomy-induced osteoclastogenesis. They could be a natural and novel treatment for postmenopausal osteoporosis.

Senile osteoporosis. Aging is another important risk factor for primary osteoporosis. At the cellular level, an increase in the level of ROS shortens the life span of osteoblasts and inhibits their formation, reduces BMSCs differentiation and stimulates the formation of damaged DNA, proteins and lipids, leading to cell apoptosis (54). On the other hand, the levels of RANKL and sclerostin (SOST, an antagonist of Wnt intracellular signal transduction) in osteoclasts are diminished (55). RANKL can stimulate ROS production. Conversely, the formation, activation and bone-resorbing function of osteoclasts also need ROS stimulation (56). When the balance between osteoblasts and osteoclasts is disordered, osteoporosis usually results.

At the molecular level, aging mainly reduces the reactivity of insulin and estrogen, which decreases bone formation and differentiation. Research shows that the level of carnosine gradually decreases with age (57). Due to carnosine's effect on glycolysis, it has been used to inhibit cell aging in cultured human fibroblasts (9). Other experiments in elderly (10-month-old) female rats found that when the bone tissues were cultured for 24 h in a medium containing ZnC (10^{-5} M), 4.5% glucose, 0.25% bovine serum albumin and antibiotics, the activity of ALP in osteoblasts was significantly increased and prolonged, thus lengthening the cell cycle. ZnC could also activate aminoacyl-tRNA synthetase, a key enzyme in the process of bone protein biosynthesis and translation, stimulate bone formation and mineralization and alleviate senile osteoporosis (47,58).

Disuse osteoporosis. Mounting evidence shows that bone disuse inhibits bone formation and increases bone resorption. These, coupled with the low dynamic and insulin-mediated gravity loading effect, eventually lead to osteoporosis (59).

It has also been observed that weightlessness damages the diaphyseal part more than the metaphyseal part in the model of hindlimb suspension in rats (60). Additionally, weightlessness or long-term physical fixation does not affect the intestinal absorption of zinc, but it partially inhibits the transfer of serum zinc to bone tissue. To compensate, zinc ions overflow from the bone leading to a decrease in zinc level in the bone matrix and cell components, which inhibits bone formation. Some researchers have suggested that inflammation from bone disuse could reflect periarticular osteopenia. This uncoupled state of bone resorption and formation also leads to periarticular osteoporosis in patients with rheumatoid arthritis (61).

In vitro, ZnC (10^{-5} M) in a culture medium with bone tissue could stimulate the proliferation and differentiation of stem cells, the production of IGF-1, TGF- β and osteocalcin and the peptization of aminoacyl-tRNA synthetase in osteoblasts, thus increasing ALP activity and aminoacyl-tRNA content of protein in bone tissues. IGF-1 stimulated by carnosine compounds could both increase bone trabecula formation and the proliferation of osteoblasts and have an anabolic effect on bone metabolism with or without bone disuse (62).

Other secondary osteoporosis. Long-term use of glucocorticoids can reduce bone formation by directly inhibiting the proliferation and differentiation of osteoblasts (inhibiting Runx2 and Collagen I) and increasing apoptosis and bone absorption by negative feedback gonadotropin secretion, or decreasing calcium absorption and increasing calcium excretion. This eventually leads to a negative calcium balance in the body, decreased bone density and secondary development of glucocorticoid-induced osteoporosis (63). A study of hydrocortisone-induced osteopathy showed that carnosine compounds prevent the increase of PTH levels and the decrease of ALP activity and increase the DNA and zinc contents. These effects prevent disordered bone metabolism and architectural deterioration (64).

In clinical studies of peripheral osteoporosis caused by autoimmune arthritis, inflammation caused by ROS oxidative stress is the key cause of osteoporosis (65,66), whereas carnosine compounds block the decrease of ALP activity induced by IL-1 β , attenuate the decrease of DNA and calcium contents, partly improve the immune response triggered by the release of matrix degradation products, inhibit inflammatory osteolysis and the loss of trabecula and collagen and alleviate the continuous destruction of bone microstructure and mechanical strength (67).

Vitamin D has extensive endocrine and autocrine functions that could promote the proliferation of osteoblasts, the differentiation and activity of osteoclasts, bone formation, absorption and coupling (68), which are essential for bone development and maintenance. Some evidence shows that 1,25 (OH) $_2$ D $_3$ is an effective promoter of osteoblasts-osteocytes transformation including promoting osteoblasts mineralization and inducing expression of bone cell markers in pluripotent stem cells (iPSop) *in vitro* (69). Notably, deficiency of vitamin D or calcium caused by various factors in the body leads to osteoporosis (69). A study by Segawa *et al* (70) observed that an oral administration of 100 mg/kg/day of ZnC for 14 days could partially increase ALP activity and calcium contents, which directly stimulated bone formation. This was independent of the change in serum mineral homeostasis, thus it could prevent the deterioration of bone tissue caused by malnutrition.

Taken together, though the pathologic process of the aforementioned types of osteoporosis vary, in terms of hormone levels, cellular response and involved signaling pathways, they all exhibit impacted cellular function, abnormal bone turnover and somewhat enhanced lipogenesis and oxidative stress, which provide targets for carnosine to protect bone. In this context and based on the current evidence, carnosine complexes are more conducive to postmenopausal osteoporosis, with a potential application value in the secondary osteoporosis.

Fracture healing. Fracture healing is a complex process based on the interaction between the inflammatory and hematopoietic stem cells and is associated with rapid osteogenic and chondrogenic differentiation of periosteal cells and continued vascular angiogenesis and ingrowth. This is subsequently followed by the remodeling of immature bone into the well-structured lamellar bone and other biochemical signal cascades (71). Animal experiments have demonstrated that macrophages are essential for both intramembranous and endochondral ossification of bone after a fracture. They contribute to the deposition of woven bone and the formation of the soft callus for endochondral ossification (72,73).

Studies have confirmed that the use of carnosine compounds could significantly promote the expression of protein components and the release of cytokines from osteoblasts in the periosteum and endosteum during bone remodeling following a fracture (7,38,74). These include bone formation stimulating factors such as PTH, IGF-1, TGF- β , OCN and 66-kDa albumin that enhance bone synthesis (75). A large amount of IGF-1 deposited in the bone matrix participates in local anabolism cooperating with PTH. The positive feedback induces bone tissues to produce more albumin, which has a synergistic effect in early and late fracture healing (76). This contributes to maintaining bone mass and bone homeostasis during bone reconstruction. The MAPKs inhibitor PD98059 or the PKC inhibitor staurosporine can completely inhibit zinc ions, thus weakening their effect on fracture healing and reducing carnosine compounds available in the body. The bioactive effect of carnosine may be partly exerted through the MAPK signaling pathway or PKC-mediated activation of albumin synthesis in bone tissue (74).

In vitro research by Hughes *et al* (77) supports this point of view. By using protein synthesis-promoting dietary supplements, the albumin, muscle content and BMD in fracture healing tissue and the expressions of IGF-1, IGF-2, actin, myosin and vascular endothelial growth factor (VEGF) mRNA in muscles were significantly increased. Ko *et al* (38) found that polaprezinc can enhance the activity of both osteoblasts and osteoclasts, thus accelerating the healing process of fractured bone.

Overall, although the specific mechanism of promoting fracture healing is unclear, there is no doubt that therapeutic or dietary supplementation of carnosine compounds benefits soft tissue repair, muscle mass recovery and bone remodeling.

Osteoarthritis (OA). OA is the most prevalent chronic joint disease, characterized by cartilage degeneration, subchondral bone osteosclerosis and synovium inflammation.

It has been proved that oral carnosine supplement could reduce the expression of IL-1 α and TNF- α in serum and synovial tissues, inhibit MMPs (MMP3, MMP13) and thrombospondin type 1 motif in the extracellular matrix, the nuclear translocation of NF- κ B p65 and finally reverse the inflammatory response specifically through the ROS/NF- κ B signaling pathway, showing a protective effect on diabetes-induced OA (29). In a canine OA model induced by anterior cruciate ligament transection (ACLT), carnosine prevented and trapped lipid peroxide 4-HNE (18), inhibiting the production of catabolites in chondrocytes and reducing the expression of inflammatory cytokines. In addition, carnosine could

markedly prevent arthritis and chondrocytes injury, through anti-inflammatory and anti-oxidation effects (78). A recent study by Lanza *et al* (79) further showed that a selected molecular synergy enhanced its biological activity and resistance, in which the carnosine-hyaluronic acid conjugate reduced oxidative damage to chondrocytes and cartilage degradation in OA.

A recent study from Busa *et al* (8) confirmed that carnosine can alleviate knee osteoarthritis pain, improve synovial protection and decrease cartilage degradation *in vivo*. In IL-1 β stimulated fibroblast-like synoviocyte cells, carnosine reduce the expression levels of cyclooxygenase-2, MMP-3 and MMP-13, ROS level and mitochondrial membrane permeability by activating the Nrf2/heme oxygenase-1 signaling pathway.

Thus, considering the aforementioned protective effects of carnosine in against cartilage degeneration, abnormal subchondral bone remodeling and synovitis, it may become a potential choice for OA treatment, particularly when chemically conjugated with some selected agent with enhanced antioxidative and anti-inflammation effects.

Bone tumors. Bone is the main target organ for tumor metastasis and the presence of bone metastases is generally a sign of poor prognosis for advanced malignant tumors. Preclinical and clinical studies show that carnosine has active antitumor and immunomodulatory effects (22) that may directly or indirectly affect the proliferation and metastasis of bone tumor cells, in which carnosine inhibits the glycolytic pathway and/or possibly participates in the regulation of biological activity mediated by mTOR, hypoxia-inducible factor α , STAT3 and MAPK (80). Primary bone tumors are highly invasive and strongly dependent on ATP produced by glycolysis. They can penetrate and destroy the bone cortex and expand into adjacent soft tissues, finally, affecting the whole bone (81). The viability of tumor cells is reduced in the presence of carnosine. Carnosine inhibits tumor growth, is anti-antigenic, improves genomic stability, reduces telomere impairment and protects cells from DNA damage induced by genotoxic substances (82).

Additionally, carnosine selectively inhibits the growth of transformed cells and ATP-dependent glycolysis activity, thus inhibiting the growth of tumor cells. Relevant studies have confirmed that carnosine activates the STAT3 signaling pathway, inhibits neuronal cell apoptosis after acute cerebral ischemia, increases the level of p27 (a cell cycle regulator) and shortens the growth cycle and proliferation of tumor cells (83,84). However, the overexpression of STAT3 activated by carnosine is related to the poor prognosis of osteosarcoma. A study by Gao *et al* (85) indicated that carnosine could inhibit the proliferation and invasion of osteosarcoma cells, promote cell apoptosis and suppress the growth and activity of tumors by mediating the Wnt3a/ β -catenin signaling pathway. Hwang *et al* (86) demonstrated that carnosine could be anti-antigenic by inhibiting the ERK/AKT/eNOS signaling pathway and MMP-2 mediated by VEGF, thus inhibiting the viability and activity of bladder cancer cells.

Further studies show that the implantation of bone metastases from malignant tumors may induce the imbalance of bone homeostasis and the change of the mechanical stress of osteoblasts, leading to osteoblastic and osteolytic lesions, thus accelerating the progression of tumor.

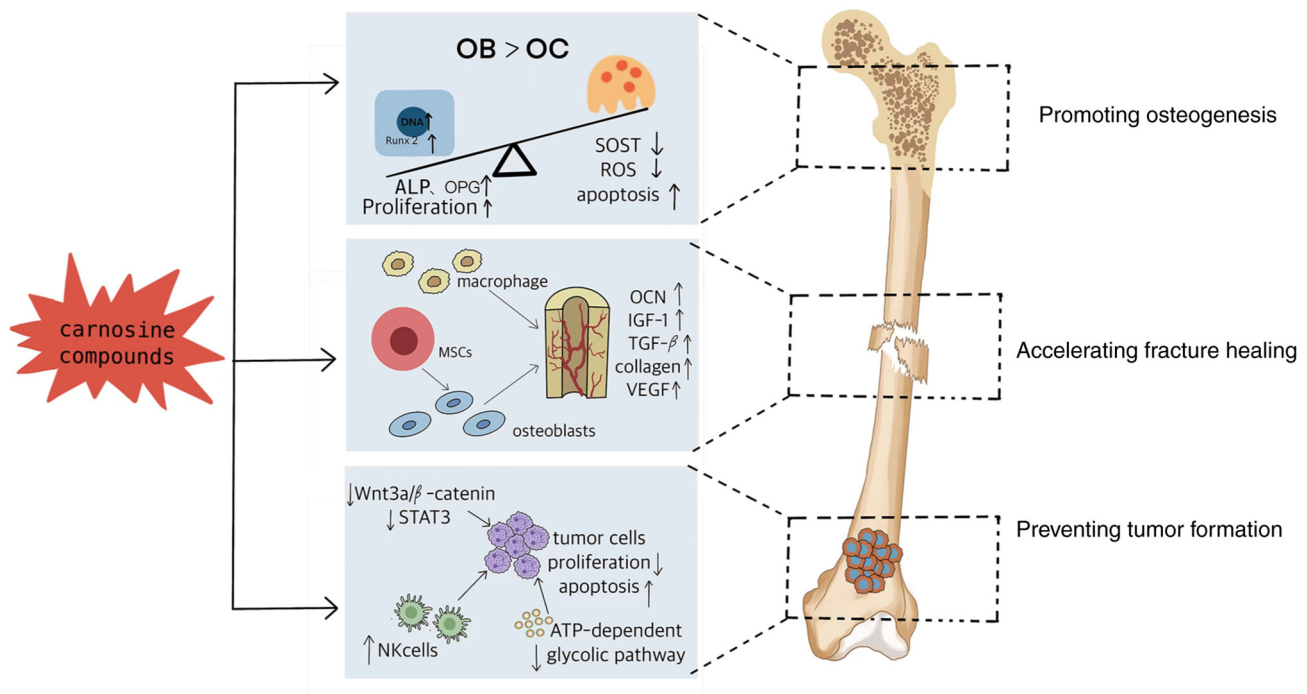


Figure 4. Effects and mechanisms of carnosine compounds on osteoporosis, fracture healing and bone tumor. Carnosine compounds promote osteogenesis by upregulating osteogenic molecules, inhibiting ROS and osteoclast function, as well as enhancing angiogenesis, thereby accelerating fracture healing, while inhibiting the proliferation and promoting the apoptosis of tumor cells. ROS, reactive oxygen species; BMSCs, bone marrow mesenchymal stem cells.

Hsieh *et al* (87) found that carnosine could inhibit the migration and invasion of human colorectal HCT-116 cells by inhibiting pro-inflammatory cytokines, oxidative stress, NF-κB and MAPK pathway activities and regulating MMPs and epithelial-mesenchymal transition related molecules such as E-cadherin, Twist-1 and MMP-9. Another important study found that the immunomodulatory action of carnosine may significantly inhibit the sympathetic nerve activity of the spleen, thereby inhibiting cancer cell proliferation by increasing NK cell activity (52).

Last but certainly not least, from the perspective of antitumor therapy options, the current evidence shows that carnosine mitigates cyclophosphamide (CTX) induced G₂/M arrest in bone marrow cells to inhibit its apoptosis and genotoxicity, downregulates the expressions of cytochrome c, Bcl-2-associated X protein (BAX) and p21, inhibits DNA damage and ROS production, protects bone marrow cells from CTX-induced oxidative stress and partly restores CTX-induced suppression of hematopoietic function (10). Additionally, the combination of carnosine and 5-fluorouracil decreases hypoxia-inducible factor 1 and multi-drug resistant protein MDR1-pg expression, overcomes the acquired resistance of some chemotherapy drugs and continually improves the anti-tumor effect of standard chemotherapy (88). Adding carnosine to adjuvant chemotherapy and radiotherapy could increase its efficiency and alleviate radiation-induced skin damage and decreased immunity. In addition, it does not have any toxic effects and could therefore act as a 'security guard' for normal tissues.

Carnosine could minimize the adverse effects of antitumor therapy in both experimental and clinical research. Exploring further the molecular mechanisms of carnosine in antitumor

growth and bone metastasis may provide a new preventive agent for bone tumor therapy.

5. Conclusions

Taken together, carnosine and its compounds could not only activate the synthesis of protein in osteoblasts, promote the proliferation and differentiation of osteoblasts and chondrocytes, but also regulate the differentiation of bone marrow macrophages into osteoclasts precursors and the activity of osteoclasts, thus enhancing the osteogenesis and promoting fracture healing, as well ameliorating the bone damage in the bone tumor (Fig. 4). Carnosine compounds should be considered a beneficial agent in the occurrence, development and process of bone reconstruction. Although further clinical experimental studies are needed to verify their benefits on bone and the mechanism are far from fully elucidated, it is reasonable to hypothesize that carnosine combined with standard anti-osteoporosis and anti-osteosarcoma therapies might improve its curative effects on a series disease with abnormal bone remodeling, as well promote fracture healing. Ultimately, it may become a promising therapeutic strategy for bone metabolism-related diseases.

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Availability of data and materials

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Authors' contributions

HY conceived and wrote the manuscript. XH conducted the formal literature search and analysis. FT made substantial contributions to conception and design. LX and FT critically revised the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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