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Review

Pathophysiological mechanism of acute bone loss after fracture

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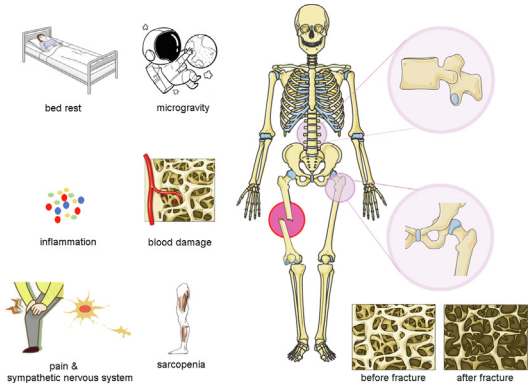
HIGHLIGHTS

- The development of scientific research in acute bone loss after fracture was elaborated in a complete and clear perspective.
- The possible mechanisms, including the new achievements in microgravity, muscle-bone crosstalk and the sympathetic nervous system, of acute bone loss after fracture were reviewed.
- Several key molecules and current treatments as well as the potential targets and promising therapies were also briefly reviewed and compared.
- The challenges we faced and the future directions are also summarized and elaborated.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Acute bone loss after fracture is associated with various effects on the complete recovery process and a risk of secondary fractures among patients. Studies have reported similarities in pathophysiological mechanisms involved in acute bone loss after fractures and osteoporosis. However, given the silence nature of bone loss and bone metabolism complexities, the actual underlying pathophysiological mechanisms have yet to be fully elucidated.

Aim of review: To elaborate the latest findings in basic research with a focus on acute bone loss after fracture. To briefly highlight potential therapeutic targets and current representative drugs. To arouse researchers' attention and discussion on acute bone loss after fracture.

Key scientific concepts of review: Bone loss after fracture is associated with immobilization, mechanical unloading, blood supply damage, sympathetic nerve regulation, and crosstalk between musculoskeletals

Abbreviations: BNST, Bed nucleus of the stria terminalis; BMSCS, Bone marrow-derived mesenchymal stem cells; BMP-2, Bone morphogenetic protein 2; CGRP, Calcitonin gene-related peptide; CNS, Central nervous system; DRG, Dorsal root ganglion; ERAS, Enhanced recovery after surgery; EP, Epinephrine; NE, norepinephrine; ERT, Estrogen replacement therapy; FRAP, Fluorescence recovery after photobleaching; GA, Gambogic amide; HGF, Hepatocyte growth factor; hMSC, Human mesenchymal stem cells; IL-6, Including interleukin-6; IGF-1, Insulin-like growth factor-1; LCS, Lacunar-canalicular system; LIPUS, Low-intensity pulsed ultrasound; LIV, Low-intensity vibrations; LPA, Lysophosphatidic acid; MGF, Mechanical growth factors; NPY, Neuropeptide Y; RANKL, NFκB ligand receptor activator; OFF, Oscillating fluid flow; OVF, Osteoporotic vertebra fractures; PTHa, Parathyroid hormone analogue; EP4, PGE2/PGE2 receptor 4; PGE2, Pro-prostaglandin E2; PGE2, Prostaglandin e2; RUNX2, Runt-related transcription factor 2; SERMS, Selective estrogen receptor modulators; SP, Substance P; SNS, Sympathetic nervous system; TAZ, Transcriptional co-activator with PDZ binding motif; TGF-β, Transforming growth factor β; VEGF, Vascular endothelial growth Factor; VIP, Vasoactive and intestinal peptide; VMH, Ventromedial hypothalamus; YAP, Yes-associated protein; α-ARs, A-adrenergic receptors; β-ARs, B-adrenergic receptors.

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Immobilization
Muscle-bone crosstalk

among other factors. Current treatment strategies rely on regulation of osteoblasts and osteoclasts, therefore, there is a need to elucidate on the underlying mechanisms of acute bone loss after fractures to inform the development of efficacious and safe drugs. In addition, attention should be paid towards ensuring long-term skeletal health.

Contents

Introduction

Acute bone loss usually occurs between 1 and 2 years after fractures, especially the first 6 to 8 weeks after acute immobilization. At this stage, the patient's bone mass sharply drops to the lowest level [1,2]. Acute bone loss after fracture is a common occurrence, especially in osteoporotic patients [3], and this clinical challenge is closed related to osteoporosis with regards to pathogenesis and clinical treatment. Osteoporosis is an age-related metabolic disease [4] that is associated with loss of bone mass, destruction of bone microstructure and increased bone fragility [5]. Therefore, studies should aim at elucidating on the pathomechanisms of osteoporosis and bone loss after fractures.

The most severe consequences of osteoporosis are fractures [6] while the most severe outcomes of bone loss after fractures are secondary fractures [7–10]. Multiple fractures in elderly osteoporotic patients are a clinical challenge. Subsequent fractures in osteoporotic patients mostly occur in different parts [11,12] and patients with fractures are also predisposed to varying degrees of osteoporosis [13–15]. Bone loss after fractures is an important reason for occurrence of re-fractures, however, the exact pathomechanisms have yet to be fully established. After fracture, bone remodeling increases in both skeletal and systemic regions adjacent to the fracture site. The increase in bone turnover near the fracture is referred to as the regional acceleratory phenomenon (RAP), while the increase in bone turnover in other regions is

referred to as the systemic acceleratory phenomenon (SAP). Both RAP and SAP enhance bone formation by osteoblasts and bone resorption by osteoclasts, however, the rate of bone resorption usually exceeds the rate of bone formation, leading to bone loss in the fractured area and systemic regions. RAP and SAP have different performances in different regions and different fractures have different characteristics, even if it is the same form of bone loss phenomenon. For instance, bone loss of the cortex, subcortex, and trabecular compartment exhibit different features [16]. Accelerated bone remodeling after fracture supports the hypothesis that fractures cause long-term systemic osteopenia, however, etiologies of bone loss after fractures are complex. Generally, immediate immobilization after injury, reduction of activity, excitement of pain-related sympathetic nerves, inflammation, destruction of blood supply and sarcopenia are potential causes of bone loss [17]. These factors are shown in Fig. 1.

Studies on pathogenesis are useful for guiding the development of approaches for early prevention, precise identification and effective treatment of acute bone loss after fracture. In this review, we comprehensively discuss the pathophysiological mechanisms of acute bone loss after fractures to provide new ideas for clinical treatment and further research.

The article will mainly review the following:

1). Elucidating on the physiological and pathological mechanisms underlying acute bone loss after fractures.

2). Summarizing the differences in bone loss after fractures, osteoporosis and bone remodeling.

3). Highlighting the drugs and systemic treatment strategies used in management of bone fractures.

Pathophysiological mechanisms underlying acute bone loss after fractures

Immediate immobilization

It is recommended that patients should stop working and rest for a period of time. Sometimes, fractures in central and important parts require absolute bed rest, otherwise the healing and functional recovery times of the fractures may be prolonged, especially if these fractures occur in the spine, limbs or other load-bearing parts [18,19]. Necessary limb immobilization is beneficial, although unscientific immobilization can lead to disuse muscle atrophy and acute loss of bone mass.

Cast or brace immobilization

Plaster fixation is one of the most common and primitive external fixation methods [20]. Technological advances in orthopaedics and industry have resulted in development of new technologies and materials, including polymer-made plaster and 3D-printed personality customized braces to replace plasters. Plaster fixation has several advantages, key among them being the use of a strong structure to support, fix, and maintain the correct bone shape as well as mechanical curve, thereby stabilizing the injured environment, and enhancing fracture healing [21].

Advances in medical technology coupled with improvement of perioperative management have enhanced the attitudes of patients and doctors towards surgery. Although the time taken for plaster fixation has significantly shortened, it is undeniable that plaster still have a place today for its low costs and wide applicability.

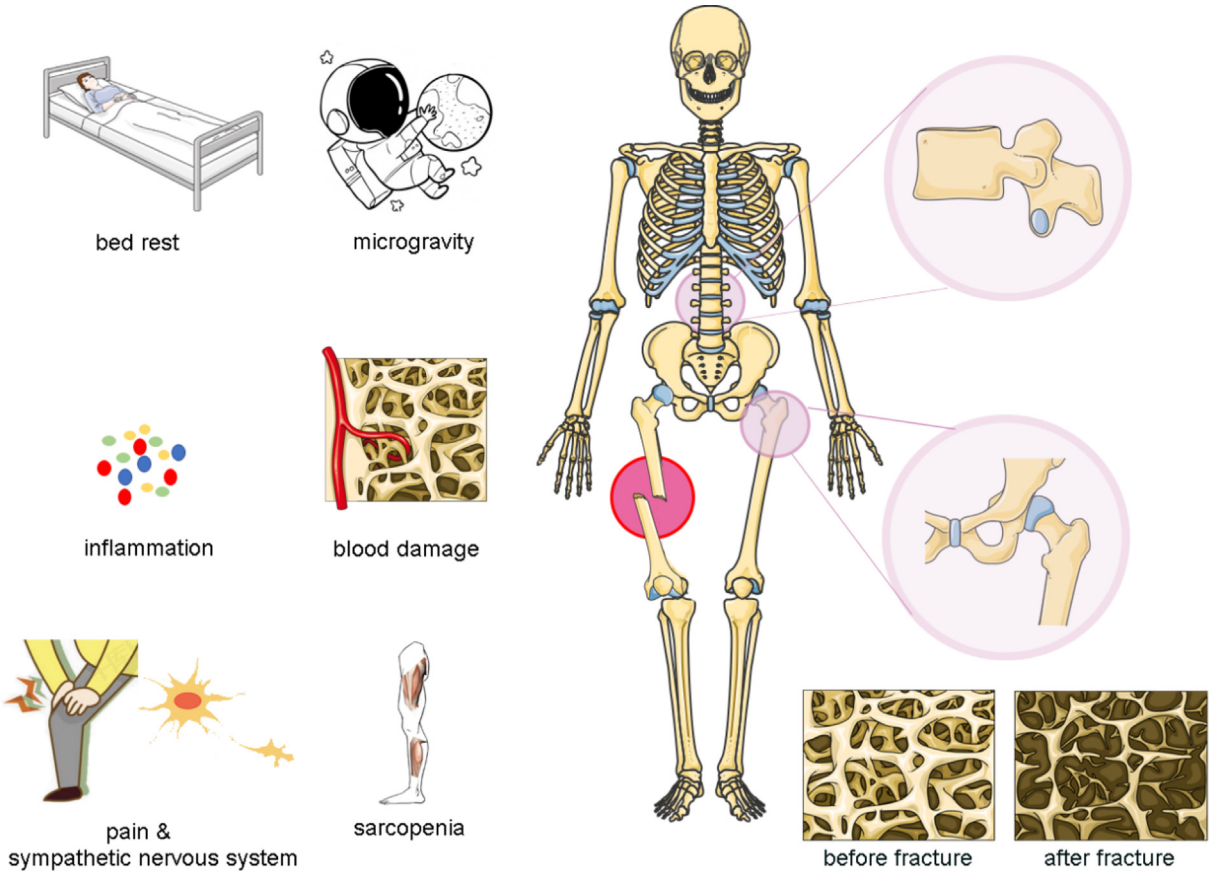

Fig. 1. Immediate immobilization after injury, reduction of activity, excitement of pain-related sympathetic nerves, and destruction of blood supply are hypothesized as the causes of bone loss in an injured area. Reduced body exercise caused by bed rest or self-cultivation, and the related changes in the crosstalk of the musculoskeletal system, represent the main reasons for loss of bone mass in other parts of the body.

Nevertheless, immediate immobilization of limbs may be a potential cause for acute bone loss after fractures.

The key molecular of unloading-induce bone loss

Wolff's law states that bone growth is affected by mechanical stimulation to optimize their structure [22–24]. The growth direction of bones, especially trabecular ones, has strong adaptability to the mechanical environment with the smallest mass and the largest mechanical efficiency. Generally, mechanical stress enhances bone strength by affecting collagen arrangement. Moreover, it upregulates the expressions of bone-derived cytokines, thereby improving the activity of osteoblasts and promoting cell proliferation as well as differentiation. Under normal circumstances, the human body is either in an upright state or in constant motion. Under gravity, human bones and muscles share the body's weight. The musculoskeletal system bears most of the physiological gravity load, which continuously stimulates the body to strengthen the bones and muscles while promoting osteoblast mobilization and osteoclast activation. This complex and mechanically mediated bone turnover is essential for maintaining healthy bone metabolism and density [25,26]. When a patient suffers from acute immobilization or plaster fixation due to fractures, trauma, hip replacement, knee replacement, spinal cord injury, and conservative treatment of intervertebral disc disease or orthopaedic traction, weightlessness (also known as microgravity or unloading) will occur, and this effect is magnified with increasing immobilization time [27].

Apart from the primary osteoporosis that is caused by aging and decreased gonadal functions, the microgravity environment has been implicated in occurrence of disuse and secondary osteoporosis. Moreover, the microgravity environment is associated with a high rate of bone loss, relative to that on the ground. It takes 6 months to 2 years to recover a patient's bone mass after fractures, which is a long period [28–30].

Mechanoreceptors

Osteocytes comprise more than 90 % of all bone tissue cells in adult bones. Almost all bone matrix surfaces are covered by osteocytic bodies and processes. The complex pore system, comprising bone lacunas and tubules, is extensively distributed in the cortical bone. Interstitial fluid in bone lacuna and tubules provides nourishment, facilitates excretion of metabolites as well as other material exchange functions and plays an important role in force perception [31]. Osteocytes are the most important mechanical receptors. Application of an external force on bone cortex generates a slight deformation on bone matrix, causing pressure differences in tissue fluids, which triggers its flow into bone tubules [32]. These changes in fluid mechanics are detected by the sensor in osteocytes, which enhances its effects. This form of oscillating fluid flow (OFF) of tissue fluids plays a role in conversion of mechanical signals into biological signals and their amplification [33].

Based on the above theory, the dynamic process of solute convection and fluid flow in the lacunar-canalicular system (LCS) of the bone can be understood [34] by using a new experimental method. This method is based on fluorescence recovery after photobleaching (FRAP) as well as simultaneous mechanical loading and imaging, and successful quantification transport of a fluorescent tracer in bone LCS. Under the influence of microgravity, flow direction of the tissue fluid in bone tubules changes from the original longitudinal to lateral or disordered direction, which is attributed to the inability to be affected by gravity [35]. At this point, mechanical signals cannot be effectively converted into biochemical ones. Moreover, under microgravity conditions, mass transfer cannot reach the deep voids under microgravity conditions, a phenomenon that results in apoptosis of bone cells and bone loss. Notably, under microgravity, there is drastic drop in mechanical signals, and this cannot be compared with the situation under normal gravity.

During the transformation of fluid flow into cellular responses in bone cells, the primary cilia of bone cells gets connected and are regulated by downstream cell responses through a Ca^{2+} entry and polycystin-2 independent signaling mechanism [36]. This allows mechanical signals to alter cell activities via tissue-specific pathways, thereby regulating homeostasis across different tissues. Upon exposures to appropriate mechanical stimulation conditions, osteogenic-related genes are upregulated in human mesenchymal stem cells (hMSC), and this process requires primary cilia [26,37]. Then, RNA-based inhibition of primary cilia downregulates bone formation-associated genes in cells, even in the presence of mechanical stimulation [27,37,38]. Under microgravity conditions, primary cilia on surfaces of osteoblasts gradually atrophy, become shorter and eventually disappear, whereas cell microtubules are depolymerized, and the cytoskeleton undergoes significant changes [38]. Additional evidences have further shown that primary cilia are closely associated with the cytoskeleton [39,40]. These findings imply that primary cilia are potential interstitial sensors of gravity (under normal or microgravity conditions) and their disappearance results in microgravity-induced osteoinhibition.

Hippo-YAP/TAZ

The Hippo signaling pathway regulates cell proliferation, apoptosis, and controls normal volume of tissues and organs as well as homeostasis of the internal environment [41]. Moreover, it is associated with other processes, including embryonic development, growth regulation, tumorigenesis as well as metastasis [42] and also plays a regulatory role in bone transduction related to mechanical load [43,44]. The Yes-associated protein (YAP) and its analogue, transcriptional co-activator with PDZ binding motif (TAZ) in its kinase chain act as response molecules during mechanical signaling [45,46]. They are subjected to extracellular mechanical stimulation and cytoskeleton activation under the influence of tension, where they are transferred from the cytoplasm to the nucleus to co-activate RUNX2 through transcription to regulate osteogenic gene expressions [47]. In addition, YAP/TAZ regulates the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) [48]. Downregulated YAP/TAZ activities were found to promote adipogenesis while their upregulation resulted in induction of MSCs to enter osteogenesis [49,50]. Furthermore, YAP/TAZ activity decreases under microgravity conditions, and this is accompanied by suppressed osteogenic differentiation of MSCs [38,47].

During the translation of mechanical signals into biochemical ones, some cytokines are involved in growth regulation and initiation of cascade reactions. This process leads to activation of nuclear transcription factors that control gene transcription of downstream molecules. The complex crosstalk between mechanical transduction and growth factors should be investigated further. Bone morphogenetic protein 2 (BMP-2), which belongs to the transforming growth factor β (TGF-β) superfamily, is involved in promoting osteogenesis [51,52]. The efficiency of BMP-2-induced osteogenic differentiation is highly dependent on cell shape, cytoskeletal tension, cell-ligand interactions and matrix stiffness [53,54]. Notably, BMP-2 activates and induces the phosphorylation of the Smad family signal transduction protein (Smad1/5/8), thereby forming a heteromeric complex [55,56]. These complexes are transferred to the nucleus where they bind target genes. However, initiation of BMP-2-induced Smad signaling is not associated with cytoskeletal tension.

In contrast, YAP/TAZ is regulated by cytoskeletal tension [57]. YAP/TAZ is mainly localized in the nucleus of cells cultured on a

stiff surface and in the cytoplasm of those cultured on a soft surface [46,58]. This indicates that YAP/TAZ is an important molecular sensor for cytoskeletal tension. Moreover, this shuttle to and from the nucleus does not depend on the BMP-2 signaling pathway. Complete osteogenic differentiation requires cytoskeletal tension-induced accumulation of YAP/TAZ in the nucleus, while translocation of YAP/TAZ in the cell enhances the activation of BMP-2 induced osteogenic genes. These two signaling pathways synergistically interact to enhance the expressions of osteogenic-related genes.

Microgravity interferes with f-actin and inhibits nuclear translocation of TAZ, thereby suppressing the osteogenic differentiation ability of BMSCs [59]. However, the addition of lysophosphatidic acid (LPA) restores the positioning of TAZ in the nucleus and promotes the secretion of osteogenic indicators. RNA-based inhibition of TAZ expression does not restore osteogenic differentiation of bone marrow mesenchymal stem cells under microgravity, even after LPA addition. This indicates that LPA can restore and retain the osteogenic differentiation ability of bone marrow mesenchymal stem cells in microgravity via the f-actin-TAZ signaling pathway. LPA has been shown to effectively reverse microgravity-induced decrease in osteogenic differentiation of MSCs via the Rock-TAZ signaling pathway [48]. Moreover, daily applications of low-intensity vibrations (LIV) can rescue the microgravity-induced decrease in nuclear YAP levels [60]. In addition, LPA treatment has also been shown to increase nuclear YAP levels, while daily applications of LIV alleviated microgravity-induced LPA-induced YAP nuclear entry.

Piezo1

Piezo1/2 protein is a novel type of mechanically sensitive ion channel protein that is closely associated with mechanical stress stimulation signals [61,62]. Piezo1 is widely expressed in various tissues and regulates the development of vital organs and important physiological processes. Piezo2 is highly expressed in neurons and plays an important role in regulating the mechanical transduction of central nervous system neurons and dorsal root ganglion neurons [63,64]. This ion channel can be directly activated by mechanical stress stimulation. The channel can also non-selectively pass through divalent ions, namely Ca^{2+}, Mg^{2+}, Mn^{2+} and Ba^{2+}, as well as monovalent alkaline ions, including K^{+}, Na^{+}, Cs^{+} and Li^{+}, among others, thereby generating local transmembrane ion flux currents [65,66]. Studies on orthopaedic diseases revealed that the Piezo1 protein is not only expressed in chondrocytes, but is also associated with osteoarthritis and bone loss [67]. Conditional absence of piezo1 in osteoblasts and osteocytes was associated with reduced cortical thickness and cancellous bone volume. Conversely, applications of piezo1 agonists significantly increased bone mass. In addition, piezo1 is associated with subchondral osteogenesis [68]. For instance, piezo1 knockout mice were found to exhibit skeletal hypoplasia and trabecular insufficiency, with adults manifesting symptoms such as osteoporosis and multiple fractures.

Microgravity changes have been implicated in induction of dynamic changes of calcium signal transduction in cells. The intracellular calcium signaling pathway, a secondary messenger that activates various cellular functions, is one of the earliest events in mechanical transduction. Piezo1 transduces mechanical signals induced by low-intensity pulsed ultrasounds into intracellular calcium ions, while an influx of Ca^{2+} acts as a second messenger to activate ERK1/2 phosphorylation and perinuclear f-actin filament polymerization, thereby regulating MC3T3-E1 cell proliferations [69]. Moreover, the Ca^{2+} signal plays an important role in regulation of RUNX2 protein stability [56]. RUNX2 plays an essential role in differentiation of mesenchymal cells into osteoblasts as well as in blocking osteoblast differentiation into adipocytes and chondrocytes. RUNX2 stability is regulated by precision and complexity, of which the TMCO1–CaMKII–HDAC4 axis is regulated in a local Ca^{2+} signal-dependent manner [70]. Bone samples from osteoporotic patients and mice exhibited significantly low levels of TMCO1, relative to healthy individuals. In addition, a lack of TMCO1 has been associated with impaired endoplasmic reticulum Ca^{2+} homeostasis, resulting in endoplasmic reticulum calcium overload, thereby affecting bone formation.

Piezo1 regulates the expressions of Wnt1 and other osteogenic-related genes in bone cells via YAP1 and TAZ [69]. Piezo1 activation can mimic the effects of mechanical stimulation in bone cells to increase bone mass. In response to mechanical stimulation, Piezo1 in osteoblasts can also regulate the expressions of different proteins in the bone matrix, including several collagens, by regulating the YAP signaling pathway. In turn, these collagen subtypes regulate osteoclast differentiation and coordinate the osteoblast-osteoclast crosstalk in the bones for controlled bone homeostasis. Once a limb breaks, the level of BMSCs that could be used for both muscle and bone regeneration decreases, while their activities are further suppressed [71,72]. These outcomes limit the sources of osteoblasts, resulting in reduced development of new bones and exacerbating the extent of bone loss.

Considering that the current in vivo animal experiment designs involve unilateral or bilateral long bone fractures, the fractures faced in clinical work are not only simple transverse fractures, but more complex comminuted fractures or multiple fractures. The fractures involve injuries that require the patient to stay in bed for a longer time or to be completely non-weight-bearing. Therefore, the current experimental results are not sufficient to deduce acute bone loss after fractures. More radical views seem to be biased towards the effects of microgravity.

Muscle injury

The musculoskeletal system is an important part of the locomotion system and a crucial factor in many exercise-related physiological and pathological processes, including assisting in breathing, protecting important internal organs and endocrine regulation [73,74]. Apart from being involved in the body's normal daily physiological activities, as a whole system, bones and muscles have a sophisticated and complex crosstalk [25,75]. This not only refers to mechanical conduction [76], but also through effective growth factors. Interactions between bones and muscles regulate various processes, including fluid circulation, cellular and molecular processes, as well as mechanical transmission [25,75]. This crosstalk is both bilateral and direct, that is, from muscles to bones and vice versa. However, due to the involvement of different physiological activities, participation of various cells and small biologically active molecules as well as soft tissues, ligaments, nerves, blood vessels and other tissues outside the musculoskeletal system, this crosstalk is more complicated and sophisticated.

Biomechanical factors

Under physiological conditions, the skeletal muscle is always in a state of dynamic equilibrium, thus, maintenance of its homeostasis requires synergistic actions involving many aspects, key among them being mechanical homeostasis. The bundle structure formed by fast-contracting muscle fibers (type II fibers), slow muscle fibers (type I fibers), collagen and motor neurons, enhances its sensitivity to mechanical loads, including fluid shear force, pulling force, and gravity among others. In addition, normal gravity physical exercises can effectively increase the mechanical load of skeletal muscles, thereby strengthening them. This phenomenon is specifically manifested in improved quality of skeletal muscles and construction of its fiber bundles [77]. In contrast, a decreased mechanical load causes a reduction in mass and volume of muscle fibers. In

addition, muscle atrophy occurs before bone changes [78,79], leading to a tilt in bone balance towards enhanced bone resorption. This occurrence is evident when analyzing bone health of astronaut groups and long-term bedridden patients.

Long-term bed rest or lack of activity is associated with the loss of body proteins, which is mainly attributed to reduced synthesis in skeletal muscles and the whole body [80,81]. The muscle is attached to the muscle insert in the bone. Mechanical stimuli, including compression, stretching, bending, torsion and shear generated by muscle movements directly transmit the mechanical load onto the bones, thereby affecting bone tissue microstructure and mass. When acting on the strain generated by the bone, these forces have important stimulating effects on osteoblasts, thereby promoting the continuous formation of osteoblasts *in situ* to increase bone mass. When skeletal muscle contraction forces decreases, the mechanical force or the load exerted on the bones also decreases, and once this stimulation is weakened, it can reduce bone formation, accelerate bone resorption and lead to bone loss or osteoporosis. The muscle contraction strength is affected by many factors, including myogenic, neurogenic, and hormonal effects. An increase in age or occurrence of disuse muscle atrophy due to limb fixation markedly reduces the diameter and number of muscle fibers. These muscle fibers are replaced by fat and collagen, a phenomenon that results in reduced muscle elasticity and volume [82,83]. In addition, a decrease in activities of enzymes that regulate muscle contraction, coupled with a reduction in the number of motor neurons, eventually reduces muscle strength and activity speed [84,85]. These factors are shown in Fig. 2.

Biochemical factors

Various bone-derived factors and myogenic factors are involved in the crosstalk between muscles and the bone, including interleukin-6 (IL-6), irisin, pro-prostaglandin E2 (PGE2), insulin-like growth factor-1 (IGF-1), mechanical growth factors (MGF), vascular endothelial growth factors (VEGF), hepatocyte growth factors (HGF), and transformation growth factors (TGF-β). There are many comparable effects associated with these cytokines, as well as their own specific effects, which are mainly reflected in muscle cell proliferation and differentiation, muscle cell repair, bone balance, and fracture repairs. The functions of specific cytokines are summarized in Table 1.

Blood supply

The bone is a highly vascularized tissue [86–88]. Blood circulation and regeneration of blood vessels in the bone are essential for bone development, growth, remodeling, and repair. Blood circulation provides a steady stream of oxygen and nutrients to the bones as well as calcium and phosphate for bone mineralization [89]. Moreover, blood is the medium in paracrine circulation between bones and muscles. The damage to the perforator vessel is inevitable during the injury. However, due to network distribution of capillaries, which has a sufficient compensatory capacity, the impact of vascular damage on fracture healing is limited. Besides, when severe open injury occurs, especially with large blood vessel damage or large segmental bone defects [90], bone regeneration will be affected [91,92]. The negative consequence of poor local blood circulation is that it cannot meet the material needs for repair and healing of injured parts. Therefore, non-union of fractures or tissue necrosis may occur in severe cases.

In summary, blood vessel formation and bone formation affect each other, be it in maintenance of bone homeostasis or in the process of fracture healing. Angiogenesis indicates the possibility of bone regeneration, and blood vessel formation disorders lead to bone regeneration disorders.

VEGF is the most important growth factor in promotion of blood vessel growth [93]. It is also a bone metabolism regulator that is mainly involved in bone formation by promoting endothelial cell proliferation and blood vessel formation. In the skeletal microenvironment, VEGF is regulated by ATF4 and it affects bone angiogenesis via endothelial sprouting from embryonic metatarsals [94]. When the bone is unloaded, VEGF secretion in osteoblasts significantly increases to promote angiogenesis and resist the negative effects of bone unloading [95]. The crosstalk between osteoblasts and endothelial cells is an important pathophysiological mechanism during osteoporosis. However, excess VEGF enhances the recruitment of TRAP and cathepsin K-positive osteoclasts [96], which may involve VEGF-C, a lymphatic growth factor, as an autocrine factor that regulates osteoclast activities [97,98]. A lack of VEGF reduces bone mass [99,100]. Osteoblast-secreted VEGF levels

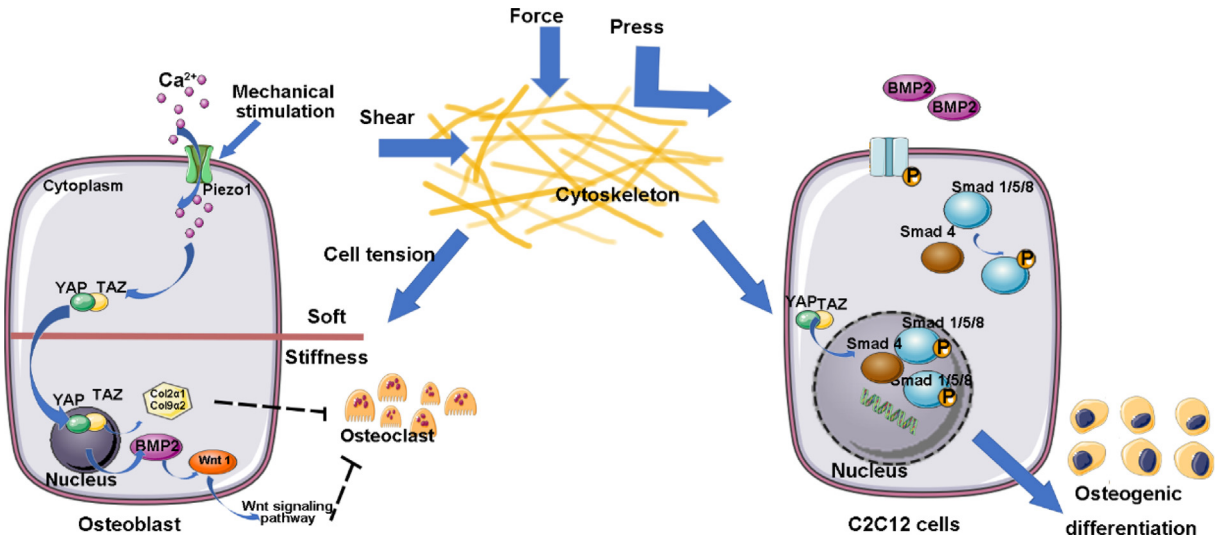

Fig. 2. YAP/TAZ is an important molecular sensor for cytoskeletal tension. Mechanoreception protein piezo1 may control the expression of Wnt1 and other osteogenic-related genes through YAP1 and TAZ. And the translocation of YAP/TAZ in the cell enhances activation of BMP-2 induced osteogenic genes. These two signaling pathways synergistically interact to enhance expression of osteogenic-related genes. Besides, BMP-2 was found to activate and induce phosphorylation of the Smad family signal transduction protein Smad1/5/8, thereby forming a heteromeric complex, which will be transferred to the nucleus where they bind to target genes.

Table 1
The role of myogenic cytokines in bone-muscle crosstalk.

Growth factors	Introduction	Origin	Function	Ref.
BDNF	A protein with neurotrophic effects, and it has the highest content in the central nervous system.	muscle	1. It plays an important role in the growth and development, survival, differentiation, and differentiation of neurons. 2. As a paracrine factor, BDNF could regulate the differentiation of satellite cells into slow vs fast myofibers. 3. Affect the synaptic connections between neurons and muscles.	[209]
CXCL-1	A small molecular weight cytokine that belongs to the CXC chemokine family. CXCL-1 is expressed by macrophages, neutrophils and epithelial cells, and has neutrophil chemotactic activity.	muscle	CXCL-1 overexpression increases muscular fatty acid oxidation with concomitant attenuation of diet-induced fat accumulation in the adipose tissue	[210,211]
FAM5C	It was originally identified in mouse brain as a gene that is induced by BMP and retinoic acid signaling	muscle	FAM5C enhances osteoblast differentiation	[212]
FGF-2	a secreted protein and belongs to the heparin-binding growth factors family.	muscle	FGF-2 not only stimulates muscle growth but also promotes intramuscular adipogenesis.	[213]
IGF-1	A peptide material necessary for the physiological effects of growth hormone, and is also called somatomedins.	muscle	1. Improve the ability of muscle cell proliferation and increase muscle abundance 2. Improve protein synthesis efficiency 3. Improve the regeneration of nerve	[214,215]
IL-5	inflammatory cytokines	muscle	IL-5 induces local accumulation of eosinophils and their release of major basic protein. The secreted proteins adhere to the muscle fiber membrane, resulting in muscle damage.	[216]
IL-6	inflammatory cytokines	muscle	IL-6 must signal in osteoblasts to favor osteoclast differentiation and the release of bioactive osteocalcin in the general circulation to increase exercise capacity.	[217]
IL-7	inflammatory cytokines	muscle	IL-7 is a novel myokine regulated both in vitro and in vivo, and it may play a role in the regulation of muscle cell development.	[218]
IL-15	inflammatory cytokines	muscle	IL-15Rα has a role in defining the phenotype of fast skeletal muscles in vivo	[219]
Irisin	A protein produced by muscles after exercise.	muscle	1.Irisin induces the expression of pro-myogenic and exercise response genes in myotubes. 2. Irisin increases myogenic differentiation and myoblast fusion by activating IL6 signaling. 3. Irisin rescues the loss of skeletal muscle mass after denervation through the activation of satellite cells and the enhancement of protein synthesis and reduction of protein degradation.	[220]
LIF	A cytokine with multiple functions, but its most important application is to maintain the undifferentiated state of mouse embryonic stem cells	muscle	LIF exerts its effect locally, stimulating the proliferation of muscle satellite cells and participating in muscle hypertrophy and regeneration to promote muscle adaptation to exercise.	[221]
Myostatin	TGF-β family	muscle	A key negative regulator of muscle repair and growth.	[222]
NT-3	Neurotrophin-3 (NT-3) is a member of the NGF family of neurotrophic factors.	muscle	NT-3 enhances axon regeneration and has potential clinical effects in preventing muscle atrophy after nerve injury.	[223]
PGE2	inflammatory cytokine	muscle	1. PGE2 directly targets muscle-specific stem cells (MuSC) through the EP4 receptor, leading to the expansion of MuSC. 2. PGE2 can enhance muscle regeneration and accelerate the repair of damaged muscles.	[224]
TGF-β1	TGFβ belongs to the TGF-β superfamily and works by binding to the type 2 TGF-β receptor (TGFBR2).	muscle	1. Participate in embryogenesis and cell differentiation and apoptosis. 2. TGFβ1 is recognized as critical negative regulator of skeletal muscle repair.	[225]
BMP-2	BMP2 belongs to the transforming growth factor-β family.	bone	BMP-2 plays an important role in the progress of bone development, bone diseases, and the repair of bone and joint injury.	[52]
DMP-1	A non-collagenous matrix protein found in dentine and bone.	bone	An acidic non-collagen protein necessary for the biomineralization of bone, cartilage, enamel, cementum and dentin.	[226]
DKK-1	DKK is a group of secreted glycoproteins, which are inhibitory molecules of Wnt pathway.	Chondrocytes, bone	Dkk-1 blocks osteoblast differentiation, induces sclerostin expression and leads to osteocyte death.	[227]
FGF-23	A kind of fibroblast growth factor.	bone	FGF-23 participate in the homeostasis of vitamin D and phosphate.	[228]
HGF	hepatocyte growth factor	bone	HGF promotes the differentiation of MSCs into osteoblasts, and plays a role in bone development, health and repair.	[229]
IGF-1	An active protein peptide substance	bone	IGF-1 promotes bone anabolism and maintains its normal structure and function.	[230]
MEPE	A non-collagenous phosphorylated glycoprotein of extracellular matrix, mainly expressed in bone tissue, dental tissue and renal proximal tubules	bone	Regulate phosphate metabolism and bone mineralization.	[231]
MGF	a splicing variant of insulin-like growth factor 1	bone	mechanical stimuli influence the physiological responses of osteoblasts by increasing the expression of MGF, which is regulated by splicing factors.	[232]

(*continued on next page*)

Table 1 (*continued*)

Growth factors	Introduction	Origin	Function	Ref.
NO	A colorless, odorless gas that is hardly soluble in water	bone	Bone cells can promote the production of NO after being stimulated by inflammatory cytokines and mechanical stress. NO directly facilitates osteoblastic differentiation.	[233]
OCN	It belongs to non-collagen acid glycoprotein and is a vitamin K-dependent calcium binding protein.	Bone, osteoblast	1. Osteocalcin (OCN) secreted by osteoblasts regulates systemic glucose and energy metabolism, reproduction, and cognition. 2. Promote bone formation and regulate bone metabolism.	[234]
OPG	A member of the tumor necrosis factor receptor family	Bone, osteoblast	1. Markers of bone turnover. 2. Promote bone formation.	[235]
PGE2	inflammatory cytokine	Bone, osteoblast	PGE2 mediates sensory nerve to control bone homeostasis and promote regeneration.	[236]
RANKL	RANKL belongs to the tumor necrosis factor family.	Bone, osteoblast	The functions of RANKL-RANK forward and reverse signaling in the regulation of osteoblast differentiation and bone formation.	[237]
SOST	A secreted protein	Bone, osteoblast	A negative regulator of bone formation.	[238]
TGF-β	A number of TGF-β superfamily that regulate cell growth and differentiation	bone	Promote cartilage and bone repair	[239]
VEGF	A highly specific vascular endothelial cell growth factor	Bone, osteoblast	VEGF is important for bone development, postnatal bone homeostasis and bone repair and regeneration.	[240]
Wnt3a	wnt3a is the main ligand of the canonical wnt signaling pathway	Bone, osteoblast	1. Canonical Wnt signaling is central to central to normal bone homeostasis. 2. Wnt3a can stimulate bone regeneration and inhibit the growth of multiple myeloma.	[241]

Abbreviation
Brain-derived neurotrophic factor，BDNF
Bone morphogenetic protein 2, BMP-2
Bone gammacarboxyglutamate protein, BGLAP
Dentin Matrix Acidic Phosphoprotein 1, DMP-1
Dickkopf-1, DKK-1
Family with sequence similarity 5, member C, FAM5C
Fibroblast Growth Factor-2, FGF2
Fibroblast growth factor-23, FGF23
Hepatocyte growth factor, HGF
Interleukin-6, IL-6
Interleukin-7, IL-7
Interleukin-15, IL-15
Insulin-like growth factor-1, IGF-1
Leukemia Inhibitory Factor, LIF
Mechano growth factor, MGF
Matrix extracellular phosphoglycoprotein, MEPE
Osteocalcin, OCN
Osteoprotegerin，OPG
Prostaglandin E2, PGE2
Receptor activator of nuclear factor-κ B ligand, RANKL
Sclerostin, SOST
Transforming growth factor β, TGF-β
Vascular endothelial growth factor, VEGF

among the elderly are low, and are associated with aging-related osteoporosis [101].

During fracture healing, VEGF is at the center of vascularization at the fracture site. VEGF-A from early osteoblasts (Osx^{+}) is essential for rapid formation of periosteal blood vessels and formation of woven bones after bone injury [102]. VEGF-A from other cells is necessary for intramedullary angiogenesis in largest cortical defects at the time of injury. In chondrocytes, FOXO1 directly binds the VEGF-A promoter and stimulates the transcriptional activities of VEGF-A [103]. This suggests that it is involved in regulation of angiogenesis in the cartilage during bone fracture healing. The SDF1/CXCR4 related pathways regulate the recruitment of endothelial progenitor cells (EPC) in tissues, which is an important prerequisite for angiogenesis at fracture sites [104].

During fracture healing, there is enhanced crosstalk between important signaling pathways, including those involving VEGF and BMP2 [105]. For instance, biglycan is significantly involved in regulation of bone homeostasis. Biglycan regulates bone cell functions by directly binding BMP2 and regulating its ability to stimulate downstream signal transduction, thereby promoting bone cell differentiation [106]. Besides, it can bind VEGF and stimulate its expressions via the TLR pathway [107]. However, specific mechanisms through which it regulates downstream of the VEGF pathway have not yet been elucidated. VEGF was found to be markedly suppressed in the callus when compared between biglycan conditionally knocked out mice with the control group seven days after the fracture [108]. At 14 days after fracture, the newly formed cartilage and braided bone in the biglycan-KO group were compared. There was less collagen formation while the degree of vascularization was low, which means the injured bone repair capacity. Expressions of NTs and their receptors were upregulated during the bone repair process. Additional treatment with recombinant NT-3 increased the mRNA levels of *BMP-2, VEGF*, and expressions of the endothelial cell marker (CD31) at the injury site, which promoted injury repair [109]. Tissue vascularization promotes bone repair. Therefore, vascularization is vital in fracture healing, and is affected by many signaling pathways as well as biomolecules.

Special mechanical changes

In physics, stress is defined as the internal force that interacts with various parts of an object when an object deforms due to external factors. This internal force is aimed at restoring the object from its deformed position to its pre-deformed position [110,111]. During fracture treatment, stress is involved in repair of fracture trauma and a reasonable use of stress can promote fracture healing. Inappropriate mechanical forces can delay fracture healing.

Stress shielding effects

The stress shielding effect suggests that the load and redistribution of stress as well as strain will occur when two or more components with elastic modulus form a loading system [112,113]. The component with higher elastic modulus will bear more load, whereas the material with a low elastic modulus bears less load, thus, there is less deformation. In fracture healing, this phenomenon is reflected in stress shunt of the fixing material to the bone. A very strong internal plate fixation will compress the blood supply under the plate, compress the periosteum, and reduce the force pressed on the bone, resulting in thinning of the cortical bone, decreased bone density, bone structure disorders, and local osteoporosis on the fixed side [114,115]. Comparable challenges have received attention in strong external fixation, artificial prosthesis implantation, and spinal pedicle screw internal fixation. Although the stress shielding effect directly hinders callus formation [116–118], its biological effects and its effects on fracture healing have yet to be fully established.

Improvement of the internal fixation material [119,120], geometric appearance of the steel plate [121], distribution and angle of the nail hole [122], and steel plate elasticity [123] are used to reduce stress shielding effects during steel plate fixation. Moreover, finite element simulation analysis and simulation experiments of in vitro specimens on multi-dimensional biomechanical machines significantly increased the application potential of virtual technologies in assessing changes in biomechanics [88,124].

Special vertebral multiple fractures

Osteoporotic vertebral fractures have different characteristics. Incidences of re-fractures after surgery are extremely high in patients with osteoporotic vertebral fractures (OVF) [125]. The most important factor is the progress of osteoporosis [126], and the direct factor is the poor rigidity and strength of the vertebral body and adjacent vertebrae. Spinal fractures are often accompanied by varying degrees of kyphosis and changes in physiological curvatures of the spine, which makes the body's center of gravity to move forward. In addition, when stress of the anterior and middle columns of the vertebral body increases, vertebral fractures are more likely to occur [127].

The thoracolumbar spine is a segment that migrates from the thoracic vertebra with less mobility to the lumbar vertebra with greater mobility [128], thus, biomechanics stress is relatively concentrated in thoracolumbar spine. Spinal fractures often occur at the thoracolumbar vertebra, especially at T11 to L2 segments. The injured vertebrae became extremely hard after surgery, leading to changes in spine biomechanics. This change is also considered to involve natural progression of degenerative lumbar spine disease, thereby accelerating intervertebral disc degeneration, intervertebral space stenosis, and vertebral body instability. This leads to changes in physiological curvatures and biomechanics of the spine, and ultimately affects the spine and fracture [129].

Neuromodulation

The central and peripheral nervous systems are jointly involved in regulation of bone development, bone metabolism, bone repair, and bone regeneration via peripheral nerve synapses and secretion of neurotransmitters. The relationship between the sympathetic nervous system and the skeleton has been studied. The sensory nervous system is associated with pain perception. A limited number of studies have reported on the association between the parasympathetic nervous system and bone metabolism. Nerve fibers in the bone tissue contain a large number of neuropeptides, including calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), substance P (SP), vasoactive and intestinal peptides (VIP). Among them, SP and CGRP exist in the sensory nervous system, whereas VIP and NPY exist in the sympathetic nervous system. Various neuropeptide receptors are distributed on human osteoblasts and osteoclasts [130]. The peripheral nervous system binds specific receptors on osteocytes and acts on specific signaling pathways to regulate osteoblasts and osteoclasts, including their proliferation, differentiation, calcification, and reabsorption pathways to achieve a balance between bone resorption and reconstruction [131–133].

Nervous system distribution

Regulation of bone mass may depend on nerve fibers that are distributed in the periosteum and bone tissue. Periosteum structures differ depending on anatomy and age. Traditionally, the periosteum is often divided into a superficial fibrous layer and a deep cambium layer, with no clear boundaries between the layers. In addition to thick collagen fiber bundles that fix the periosteum and ligaments, the periosteum is rich in blood vessels and cells, including osteoprogenitor cells, osteoblasts, osteoclasts, and vascular endothelial cells. The periosteum is also rich in innervation, including myelinated and unmyelinated sensory nerve fibers, mainly unmyelinated nerve fibers. Sympathetic nerves in the bone, especially noradrenergic nerves, are mainly distributed at the location near blood vessels, and the most abundant are in areas with high osteogenic activities, such as near the epiphyseal growth plate. Mature osteoblasts and osteoclasts can form synapses with appropriate axons. The extension of sympathetic nerve axons to osteoblasts and osteoclasts is subject to dynamic neuroregulation of local bone metabolism [134].

The effects of sympathetic nervous system

Under physiological and pathological conditions, the sympathetic nervous system can directly or indirectly affect bone remodeling, and its functional disorders can also affect bone metabolism to result in various bone diseases. When the sympathetic nervous system is excited, the adrenal medulla secretes epinephrine (EP) and norepinephrine (NE). NE is the most common transmitter released by the sympathetic postganglionic fibers. There are various adrenergic receptors on membranes of human osteoblasts and osteoclasts. These receptors are divided into two: α-adrenergic receptors (α-ARs) and β-adrenergic receptors (β-ARs). The β-ARs are closely associated with calcaneal metabolism. When the sympathetic nerve is excited, plasma norepinephrine levels increase, osteogenesis is inhibited, and bone resorption is promoted through β-adrenergic receptors in the bone to reduce bone mass. The effect of sympathetic nervous system on bone metabolism are briefly outlined in Fig. 3.

A previous study found that sympathetic excitability of an astronauts' muscles was higher after a flight test than before the flight, and serum NE levels were also higher after the flight test than before [135]. In addition, the peripheral sympathetic nervous system in bed nucleus of stria terminalis -ventromedial hypothalamus (BNST-VMH) neural circuitry is involved in regulation of stress-induced bone loss [136]. The bone marrow adipose tissue is a fat depot and an endocrine tissue, and is also regulated by sympathetic nerves for orderly energy metabolism and endocrine regulation [137,138]. Bone and bone marrow adipose tissues may also

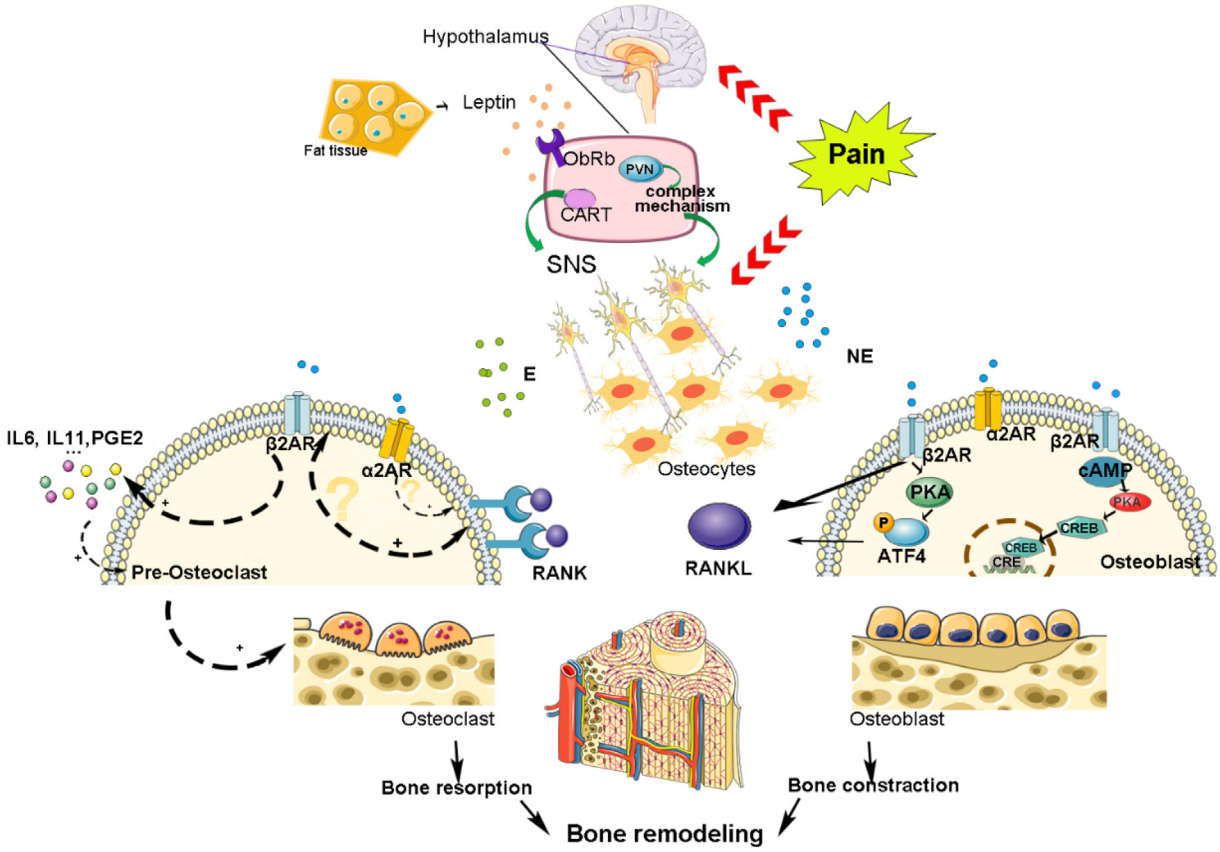

Fig. 3. As the target organ of the nervous system, the bone is innervated by the sympathetic nervous system. Specifically, the brainstem and hypothalamus integrate internal and external signals, and noradrenergic fibers are present in the periosteum, bone, and bone marrow. The extension of sympathetic nerve axons to osteoblasts and osteoclasts is the anatomical basis for the dynamic neuroregulation of bone metabolism. After fracture, the enhancement of sympathetic tone, caused by fracture pain, increases the level of norepinephrine, which will bind to specific receptors (such as β_2R) on osteoblasts or pre-osteoclast. The osteoblasts differentiation and osteoclasts maturation were regulated though specific signaling pathways to achieve the balance between bone resorption and bone reconstruction.

modulate bone metabolism under precise regulation of the sympathetic nervous system. The crosstalk between the two has been elucidated [139]. The central nervous system, especially the sympathetic nervous system (SNS), regulates bone mass [140,141], however, the specific signaling pathways or key molecules have not yet been clearly determined.

A crosstalk between the sympathetic nervous system and leptin has also been found in bone metabolism [142]. Leptin acts on neurons in the ventral midline of the hypothalamus and stimulates NE sympathetic nerve fibers to release NE [136]. The released NE specifically interacts with β2-AR on the osteoblasts [143], thereby inhibiting the activities of osteoblasts and bone formation. Therefore, leptin regulates bone remodeling via at least two different antagonistic pathways. On one hand, the sympathetic nerve signal of Adrb2 promotes osteoclast differentiation [144] while on the other hand, realization of this sympathetic nerve function is controlled by phosphorylation of ATF4 [141].

Administration of low doses of leptin in ovariectomized osteoporotic female rats effectively reduced estrogen deficiency-associated trabecular bone loss and structural changes [140]. Thus, leptin may play an important protective role in bone metabolism by inhibiting bone resorption. This could be because leptin is a potential inhibitor of bone formation via the central nervous system and also enhances bone metabolism via peripheral mechanisms.

Given that the sympathetic nerve system is plastic [145], pain will result in sympathetic nerve excitement, whose hyperactivity may amplify pain signals [146]. In ovariectomized mice, destruction of sympathetic nerve fibers was associated with a reduction in their movements after the fracture, which affected bone tissue remodeling in the latter stages of fracture repair. However, the destruction had no effect on pain-related processes [147]. Our current research shows that fracture pain may contribute to systemic bone loss by activating sympathetic nerves through the central nervous system (Unpublished data).

Pain and sensory nervous system

Pain is a central feature in tissue damage. The pain after fracture is very severe [148], and orthopaedic surgery is considered to be the most painful operation [149]. Fracture-associated pain may be caused by a combination of local inflammation, cytokine release, neurotrophic factor release, sympathetic axon sprouting, abnormal activation of DRGs, and glial cell activation [150].

NGF-TrkA

The combination of NGF and its receptor (TrkA) is one of the main molecular signals that produce bone pain. Most of the sensory nerve fibers innervating the bone express TrkA+ [151,152]. TrkA can guide sensory nerve axons to the initial ossification site. In addition, NGF produced by osteochondral progenitor cells activate TrkA to act as a skeletal neurotrophic factor [153]. Therefore, NGF-related signaling pathways play an important role in coordinating sensory innervation, vascularization, and ossification of long bone development, which is essential for normal primary and secondary ossification.

Under physiological conditions, mechanical signals upregulate NGF expressions in osteoblasts, which in turn activate TrkA sensory nerves, leading to regulation of Wnt/β-catenin signal transduction and enhanced bone formation [154]. Piezo2, a

mechanically gated ion channel, aids the sensitivity and responsiveness of bone afferent neurons to harmful/normal mechanical stimuli [52,63]. Piezo2 knockout animals exhibited reduced firing frequencies and inhibited NGF-induced sensitization of bone afferent neurons [155]. The combination of NGF and its receptor (TrkA) forms the main molecular signal that produces bone pain. Under pathological conditions, soft tissue trauma leads to high expressions of NGF and NGF-responsive axon invasion occurs before osteoclast differentiation. Cartilage and bone formation were significantly delayed when the sciatic nerve was surgical removed [156]. Single-cell sequencing showed that the signal of prechondral cells shifted from TGF-β to FGF activation after denervation. Therefore, activation of the FGF signal may indicate inhibition of bone and cartilage differentiation of MSCs.

The use of opioids or NSAID drugs during fracture healing can effectively relieve pain, however, there is the potential harm of impeded fracture healing. Autophosphorylation of TrkA receptors can be prevented by using specific neutralizing antibodies to block NGF/TrkA signaling, thus, the subsequent signaling cascade is not activated, which eliminates pain transmission without delaying fracture healing [157]. This was also confirmed by Lilian *et al.* [158], who found that blocking NGF activities by inhibiting TrkA reduced pain in an osteoarthritis rat model. The possible reasons are the asymmetry of weight-bearing caused by the irregular wear of articular cartilage in patients with osteoarthritis, and the standing pain caused by the combination of pain and allergies. In this process, NGF/TrkA plays a crucial role in establishment and amplification of pain sensations. However, there are different points of view with regards to the fact that the use of the TrkA agonist (gambogic amide (GA)) can increase NGF-TrkA signaling in the bone, thereby enhancing bone adaptation to mechanical forces [159]. This results in increased load-induced bone formation and anabolic signaling without inducing significant thermal pain or mechanical hyperalgesia. Skeletal sensory nerves play a role in pain and fracture healing, therefore, any pharmacological method that changes sensory nerve functions may affect bone anabolism.

Pge2-EP4

Prostaglandin E2 (PGE2) is an inflammatory factor [160,161] and a neuromodulator that alters neuronal excitability [162,163]. Excess PGE2 activates sensory neurons and mediates pain hypersensitivity by binding PGE2/PGE2 receptor 4 (EP4) receptors. Moreover, PGE2 is a multifunctional regulator of bone metabolism and the intra-skeletal sensory regulates bone homeostasis via the concentration of PGE2 in the bone [164].

The processes by which PGE2 regulates bone homeostasis include regulation of bone resorption and bone formation, that is, by up-regulating the expressions of NFκB ligand receptor activator (RANKL) to promote osteoclast differentiation and bone resorption [165]. When bone density decreases, the PGE2 secreted by osteoblasts increases, thereby inhibiting sympathetic nerve activities via the central nervous system and promoting bone formation [161,166,167]. The use of bone-targeted prostaglandin E2 receptor 4 agonists also increased the therapeutic effects of bisphosphonates in a mouse model of severe osteogenesis imperfect [168].

PGE2 is also involved in pain processes in diseases such as osteoarthritis and rheumatoid arthritis [166]. For patients with low back pain, the increase in PGE2 in the porous endplate stimulates sensory nerves, leading to spinal cord pain [167,169]. The use of low-dose celecoxib can inhibit endplate porosity, sensory innervation, as well as spinal pain and allergies [170].

Inflammation

Fractures, like any other trauma or injury, are accompanied by inflammatory responses that play important roles in repair processes. An association between chronic/systemic inflammation and bone loss has been reported. However, the impact of the inflammatory state on bone fracture has not been fully elucidated.

At 1 day after injury, the secretion of inflammatory factors significantly increases, remain high for 2 weeks, and begin to decline at 3 to 4 weeks after injury. Mice with multiple fractures [171], older mice [14], and male mice [172] tend to exhibit high levels of inflammation and corresponding bone loss. TNF-α, IL-1, and IL-6 are common pro-inflammatory cytokines that directly activate osteoclasts or increase osteoclast differentiation by inducing osteoblasts and osteoblasts to produce RANKL. In addition, pro-inflammatory cytokines suppress the production of bone matrix by enhancing the secretion of sclerostin (SOST) [173] or Dickkopf-related protein 1 (DKK1) [174]. Among them, IL-6 affects bone metabolism by regulating osteoclast and osteoblast development as well as function. IL-6 stimulates the differentiation of osteoclast precursor cells into active and mature osteoclasts with concomitant bone loss following OVX [175] or unloading [176] or upon aging [177]. The use of IL-6-neutralizing antibodies [176] or IL-6 gene knockout [178] reduced the generation of osteoclasts and alleviated bone loss.

The current research focuses on the detection of the concentration of inflammatory factors. There is no definite evidence to identify the relationship between inflammation and bone loss after fractures, but existing research results suggest that increased concentrations of pro-inflammatory cytokines may trigger increase the bone loss. There is a need to investigate the dynamic changes in inflammatory factor concentrations to identify the inflammatory factors that have the greatest impact on bone remodeling, and to inform the development of appropriate therapeutic approaches.

Therapy

Active drug interventions

The pathological mechanism of acute bone loss after fracture is mainly associated with osteoporosis. Therefore, standard anti-osteoporosis drugs should be actively used after fractures. These drugs alleviate pain, inhibit acute bone loss, reduce progressive bone loss, and restore the bone mass to the level before the injury, thereby reducing re-fracture incidences. Drug interventions are mainly divided into basic drug therapy and anti-osteoporosis drug therapy.

Calcium and vitamin D

The bone is a calcium-rich tissue [179], accounting for 99 % of the body's calcium. Calcium is necessary for fracture callus mineralization, thus, during the fracture healing process, the demand for calcium is more than usual. When dietary calcium supply does not meet the calcium requirements for callus mineralization, more calcium is mobilized from distal bones to ensure adequate bone repair [180]. Therefore, it is important to timely replenish calcium to enhance its absorption.

Calcium and vitamin D [181,182], as the basic treatment drugs for osteoporosis, are routinely used in the treatment of osteoporosis patients, with the purpose of promote bone formation, mineralization and reduce the risk of fractures. The drugs can also be used in combination with other anti-osteoporosis drugs during fracture treatment. The recommended calcium supplement dose should not be less than 1000 mg/d for adults [183], and it should be taken continuously for at least half a year, with vitamin D (800–1200 IU/d) supplementation [183–185]. Although other types of vitamins have also been studied [186–188], we will not discuss them here because of inconclusive findings regarding them.

Anti-osteoporosis drugs

Anti-osteoporosis drugs are divided into anti-bone resorption drugs (bisphosphonate drugs, estrogen, selective estrogen receptor modulators, and RANKL inhibitors) and bone formation drugs (thyroid parathyroid hormone analogs).

Estrogen and SERMs

Estrogen has a wide range of physiological effects, including skeletal system maturation and bone mass maintenance [189,190]. Postmenopausal osteoporosis is significantly associated with declining estrogen levels. Estrogen replacement therapy (ERT) can inhibit bone turnover, prevent bone loss, and increase bone density of the vertebral body and hip [191]. Notably, ERT or estrogen-progesterone hormone replacement therapy (HRT) are usually prescribed for postmenopausal osteoporotic fracture patients. Selective estrogen receptor modulators (SERMs) can selectively act on target organs of estrogen, combine with different forms of estrogen receptors, reduce bone resorption, and prevent bone loss. However, SERMs and estrogen drugs are not recommended for acute phases of fractures.

Calcitonin

Calcitonins inhibit the biological activities of osteoclasts, reduce the number of osteoclasts, and have good therapeutic effects on acute bone loss and pain after osteoporotic fractures [192,193]. Some commonly used drugs include salmon calcitonin and eel calcitonin.

Bisphosphonates

Bisphosphonates can specifically bind hydroxyphosphatidite in the bone and inhibit bone resorption by suppressing osteoclast activities [194,195]. This increases bone density of the lumbar spine and hip while reducing re-fracture incidences. Currently, bisphosphonates are the most successful and commonly used drugs for osteoporosis treatment, especially osteoporosis that is characterized by bone loss and bone structure destruction. Some commonly used drugs include alendronate sodium, risedronate sodium, zoledronic acid, and ibandronate sodium.

RANKL inhibitors

RANKL inhibitors, such as denosumab, inhibit the activation of the osteoclast RANK signaling pathway by combining with RANKL, thereby suppressing ostoclasst activation and maturation, and reducing bone loss [196,197].

Parathyroid hormone analogue

Parathyroid hormone analogues (PTHa), mainly teriparatide [198] and abaloparatide [199], stimulate osteoblast activities, increase osteoblast secretion of collagen, enhance bone matrix formation as well as mineralization, promote bone formation, and improve bone remodeling [200].

There are differences between the healing process of fractures and the pathophysiological mechanisms of bone reconstruction. The factors influencing bone reconstruction do not necessarily affect fracture healing. It has been reported that bisphosphonates have no effects on intrachondral osteogenesis but have an effect on intramembranous osteogenesis [201]. Therefore, when deciding on a treatment plan, various considerations are required.

Furthermore, drugs that affect bone regeneration, including hormones, diuretics, psychiatric drugs, and chemotherapeutic drugs should be adjusted according to the condition. Importantly, applications of anti-osteoporosis drugs should strictly follow the indications and contraindications because excess use will cause overcorrection, leading to gastrointestinal reactions, allergies, headaches, urinary tract stones, and other side effects.

Combined therapy

The degrees of bone loss among patients after fractures differ, and personalized treatment is needed. Combined treatment may achieve better results with the most minimal side effects [201–203]. However, not every fracture patient requires anti-osteoporosis medications. Therefore, clinicians should formulate an individualized treatment plan based on the degree of osteoporosis, the severity of injury, and the overall state of the patient, combined with indications, contraindications, and drug safety.

Integrated management

Effective immobilization is necessary, whether surgical treatment or conservative treatment. Scientific management and education during the immobilization period are needed [21,204,205]. Identifying high-risk patients and commencing active intervention as early as possible can significantly eliminate the influence of unfavorable factors. High-risk factors include aging, women, diabetes, high blood pressure, severe fractures, multiple fractures, long-term use of corticosteroids or other drugs, and a history of previous fractures [11,12,206]. In the perioperative period of fracture patients, it is suitable to apply an effective combination of a personalized treatment plan and modular multidisciplinary cooperation, as well as active early rehabilitation training [207,208].

Conclusion and future directions

Rapid bone loss after fracture is increasingly being studied. Its pathophysiological mechanisms are complicated and involves multiple tissues, organs and systems. In addition, the complexity of bone loss after fracture is not only due to the complex crosstalk between key molecules or signaling pathways, but is also closely associated with pathological changes caused by the fracture itself and osteoporosis progression. Fracture patients with acute bone loss require special attention because of increased risk of secondary fractures. The advantages and disadvantages of the existing drug therapy regimens suggest that there is a need to proceed with caution during treatment, implying the need for further research on bone loss.

CRediT authorship contribution statement

Xuan-Qi Zheng: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Jie Huang:** Methodology, Formal analysis, Investigation, Writing – original draft. **Jia-liang Lin:** Methodology, Formal analysis, Investigation, Writing – original draft. **Chun-Li Song:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

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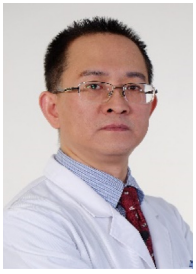

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