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Exploring the Cellular and Molecular Mechanisms Underlying Heavy Metal-Induced Osteoporosis: The Role of Cadmium, Lead, and Zinc in Disrupting Bone Metabolism in *Peromyscus leucopus*

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ABSTRACT

Heavy metal exposure is a recognized risk factor for skeletal disorders, including osteoporosis. This study investigates the mechanisms by which cadmium, lead, and zinc contribute to osteoporosis in *Peromyscus leucopus* (white-footed mouse), focusing on cellular and molecular disruptions that affect bone metabolism. Cadmium and lead, known toxicants, impair bone health through mechanisms such as inhibition of osteoblast activity, enhancement of osteoclast-mediated bone resorption, disruption of calcium homeostasis, and induction of oxidative stress. These metals interfere with essential signaling pathways, including RANK/RANKL/OPG, promoting an imbalance favoring bone resorption. Conversely, zinc, though beneficial at physiological levels, can exacerbate bone loss at higher concentrations due to oxidative stress induction and dysregulation of matrix metalloproteinases (MMPs). The study employs histological analysis, gene expression profiling, and biochemical assays to assess changes in bone structure, turnover markers, and cellular stress responses in metal-exposed mice. Findings indicate significant alterations in bone density and microarchitecture associated with elevated oxidative damage and impaired calcium metabolism. Heavy metal exposure also modulates critical regulatory pathways, shifting the balance between bone formation and resorption. These insights suggest that heavy metal exposure accelerates osteoporosis development through combined effects on osteoblast and osteoclast function, oxidative stress, and disrupted signaling pathways. Understanding the complex interactions between heavy metals and bone metabolism is essential for developing prevention and treatment strategies for heavy metal-induced osteoporosis. This research highlights the need for environmental and public health interventions to reduce exposure to harmful metals, thereby potentially decreasing the prevalence of osteoporosis in exposed populations.

Keywords: bone metabolism, cadmium, heavy metal exposure, osteoporosis, oxidative stress, signaling pathways, zinc

1 INTRODUCTION

Osteoporosis is a pervasive chronic skeletal disorder characterized by a progressive decline in bone mass and the deterioration of bone tissue architecture, culminating in an elevated risk of fractures. This condition primarily affects older adults and postmenopausal women due to the interplay of hormonal changes, aging, and lifestyle-related factors such as insufficient physical activity and poor nutrition. However, there is a growing recognition that environmental exposures, particularly to heavy metals, may also significantly contribute to the development and progression of osteoporosis. Unlike the well-established risk factors, the role of environmental contaminants in bone health has not been fully elucidated, despite mounting evidence suggesting that heavy metals such as cadmium, lead, and zinc can profoundly disrupt bone metabolism. These metals are ubiquitous in polluted environments, including industrial areas, agricultural regions where pesticides are used, and areas affected by mining activities, leading to their accumulation in air, water, soil, and, consequently, the food chain. The widespread prevalence of these metals in the environment raises concerns about their potential impact on skeletal health at the population level [1,2].

Among various animal models used to study the ef-

fects of environmental toxins on health, *Peromyscus leucopus*, or the white-footed mouse, offers several advantages for investigating the impact of heavy metal exposure on bone metabolism. This species exhibits physiological and metabolic similarities to humans, particularly in terms of bone remodeling processes, making it a relevant model for understanding how environmental factors influence human skeletal health. Moreover, the white-footed mouse's sensitivity to metal toxicity provides a valuable system for examining the molecular and cellular pathways disrupted by heavy metal exposure, which could be directly related to human health outcomes. Given these attributes, *Peromyscus leucopus* serves as an ideal organism to study the pathophysiological mechanisms underlying heavy metal-induced osteoporosis [2, 3].

Cadmium is one of the most well-documented heavy metals with toxic effects on the skeletal system. It exerts its deleterious influence primarily by disrupting calcium metabolism, which is crucial for maintaining bone mineral density (BMD) and overall bone quality. Cadmium exposure inhibits osteoblast function, reducing bone formation, while simultaneously stimulating osteoclast activity, thereby promoting bone resorption. These processes collectively result in a net loss of bone mass and compromised bone strength. Similarly, lead is known to interfere with calcium signaling pathways, disrupt osteoblast and osteoclast function, and induce oxidative stress within bone cells. These mechanisms contribute to reduced BMD and impaired bone quality, factors that elevate the risk of fractures. While zinc is an essential trace element required for normal bone growth and mineralization, excessive exposure has been associated with adverse skeletal effects. At elevated concentrations, zinc may disturb bone homeostasis by inhibiting osteoblastic activity or enhancing osteoclastic resorption, thereby potentially contributing to bone loss.

Understanding the cellular and molecular mechanisms by which cadmium, lead, and zinc contribute to osteoporosis is crucial for developing targeted therapeutic strategies and preventative measures. The current study aims to investigate the specific pathways through which these metals induce bone deterioration in *Peromyscus leucopus*. It will focus on key biological processes, including oxidative stress, disruption of calcium homeostasis, and alterations in signaling pathways involved in bone remodeling. By examining bone turnover markers, changes in bone microarchitecture, and gene expression patterns associated with bone homeostasis, the study seeks to provide a comprehensive picture of how heavy metal exposure affects skeletal health. These findings could have significant implications not only for understanding the pathogenesis of osteoporosis but also for informing public health policies aimed at minimizing heavy metal exposure and mitigating its impact on bone disease [4, 5].

2 EFFECTS OF HEAVY METALS ON BONE METABOLISM

Heavy metals affect bone metabolism through complex interactions involving various cells, signaling pathways, and systemic effects. The effects on osteoblasts, osteoclasts, and osteocytes—key players in bone remodeling—vary significantly between cadmium, lead, and zinc.

2.1 Cadmium

Cadmium is a highly toxic heavy metal with no known beneficial biological function in humans, and its toxicological impact on skeletal health is well-established. The metal's detrimental effects on bone tissue are mediated through multiple pathways that disrupt bone remodeling processes, leading to a net loss of bone mass and compromised bone strength. The primary mechanisms through which cadmium exerts its toxic effects include the inhibition of osteoblast function, enhancement of osteoclast activity, and disruption of calcium homeostasis, each of which plays a crucial role in the pathophysiology of cadmium-induced bone disease.

2.1.1 Inhibition of Osteoblast Function

Osteoblasts are specialized cells responsible for the synthesis and mineralization of bone matrix, and any interference with their function can significantly impair bone formation. Cadmium exposure is known to impede the differentiation and activity of osteoblasts, resulting in reduced bone formation. Specifically, cadmium disrupts key processes essential for osteoblast function, including the production of collagen, which forms the organic scaffold of bone tissue. Additionally, cadmium reduces alkaline phosphatase activity, an enzyme crucial for the mineralization process, and downregulates the expression of bone matrix proteins such as osteocalcin, which are necessary for maintaining bone quality and strength. The net effect of these disruptions is a reduction in bone formation, contributing to the development of osteoporosis.

2.1.2 Enhanced Osteoclast Activity

In addition to inhibiting bone formation, cadmium promotes bone resorption by enhancing the activity of osteoclasts, the cells responsible for bone degradation. This effect is mediated through the modulation of the receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin (OPG) signaling pathway, which plays a central role in regulating osteoclastogenesis. Cadmium exposure has been shown to upregulate the expression of RANKL, a key factor that promotes osteoclast differentiation and activation, while simultaneously downregulating OPG, a decoy receptor that inhibits RANKL-induced osteoclastogenesis. The resulting increase in the RANKL/OPG ratio shifts the balance toward enhanced osteoclast activity, leading to increased bone resorption and accelerated bone loss.

Table 1. Effects of Heavy Metals on Bone Metabolism in *Peromyscus leucopus*

Heavy Metal	Mechanism of Action	Impact on Bone Cells	Resulting Bone Condition
Cadmium	Disrupts calcium metabolism, inhibits osteoblast function, stimulates osteoclast activity	Reduced bone formation, increased bone resorption	Decreased bone mineral density, compromised bone strength
Lead	Interferes with calcium signaling, disrupts osteoblast/osteoclast function, induces oxidative stress	Impaired bone remodeling, increased oxidative damage	Lower bone mineral density, elevated fracture risk
Zinc	Inhibits osteoblastic activity at high concentrations, enhances osteoclastic resorption	Disruption of bone homeostasis, altered bone cell function	Potential bone loss, compromised bone quality

Table 2. Pathophysiological Mechanisms Associated with Heavy Metal-Induced Osteoporosis

Biological Process	Heavy Metal Involvement	Mechanistic Details
Oxidative Stress	Cadmium, Lead	Induction of reactive oxygen species, damage to bone cells, alteration in cellular signaling
Calcium Homeostasis	Cadmium, Lead	Interference with calcium absorption and signaling pathways, disrupted osteoblast/osteoclast balance
Signaling Pathways	Cadmium, Lead, Zinc	Alterations in pathways such as *RANK/RANKL/OPG*, *Wnt/β-catenin*, and *MAPK*, which regulate bone remodeling
Bone Turnover Markers	Cadmium, Lead, Zinc	Changes in levels of serum markers such as osteocalcin, alkaline phosphatase, and collagen degradation products

2.1.3 Disruption of Calcium Homeostasis

Calcium homeostasis is critical for maintaining skeletal integrity, and cadmium disrupts this balance by interfering with both intestinal absorption and renal reabsorption of calcium. Cadmium competes with calcium ions during intestinal absorption, thereby reducing the amount of calcium available for bone mineralization. Moreover, cadmium's nephrotoxic effects impair the kidney's ability to reabsorb calcium, leading to increased urinary excretion of calcium and resultant hypocalcemia. In response to decreased serum calcium levels, the parathyroid gland secretes parathyroid hormone (PTH), which acts to restore calcium levels by stimulating osteoclast activity to release calcium stored in bones. This compensatory mechanism, while aimed at maintaining systemic calcium levels, paradoxically exacerbates bone resorption and contributes further to bone loss associated with cadmium exposure [6].

The cumulative effect of these mechanisms underscores cadmium's role as a potent disruptor of bone remodeling, promoting a state in which bone resorption outpaces bone formation, thereby increasing the risk of osteoporosis. Understanding the molecular and cellular basis of cadmium's impact on bone health is essential for developing targeted therapeutic interventions aimed at mitigating its toxic effects on the skeletal system.

2.2 Lead

Lead exposure has been recognized as a significant environmental factor that negatively impacts bone metabolism, contributing to the development of osteoporosis. The toxic effects of lead on bone health are mediated through various mechanisms that disrupt the delicate balance of bone remodeling, ultimately favoring bone resorption over bone formation [7]. The primary pathways through which lead exerts its harmful effects include the impairment of osteoblast activity, promotion of osteoclast differentiation, and interference with the metabolism of calcium and vitamin D, all of which contribute to compromised bone integrity.

2.2.1 Impairment of Osteoblast Activity

Osteoblasts are essential for the deposition of new bone tissue, as they are responsible for the synthesis and mineral-

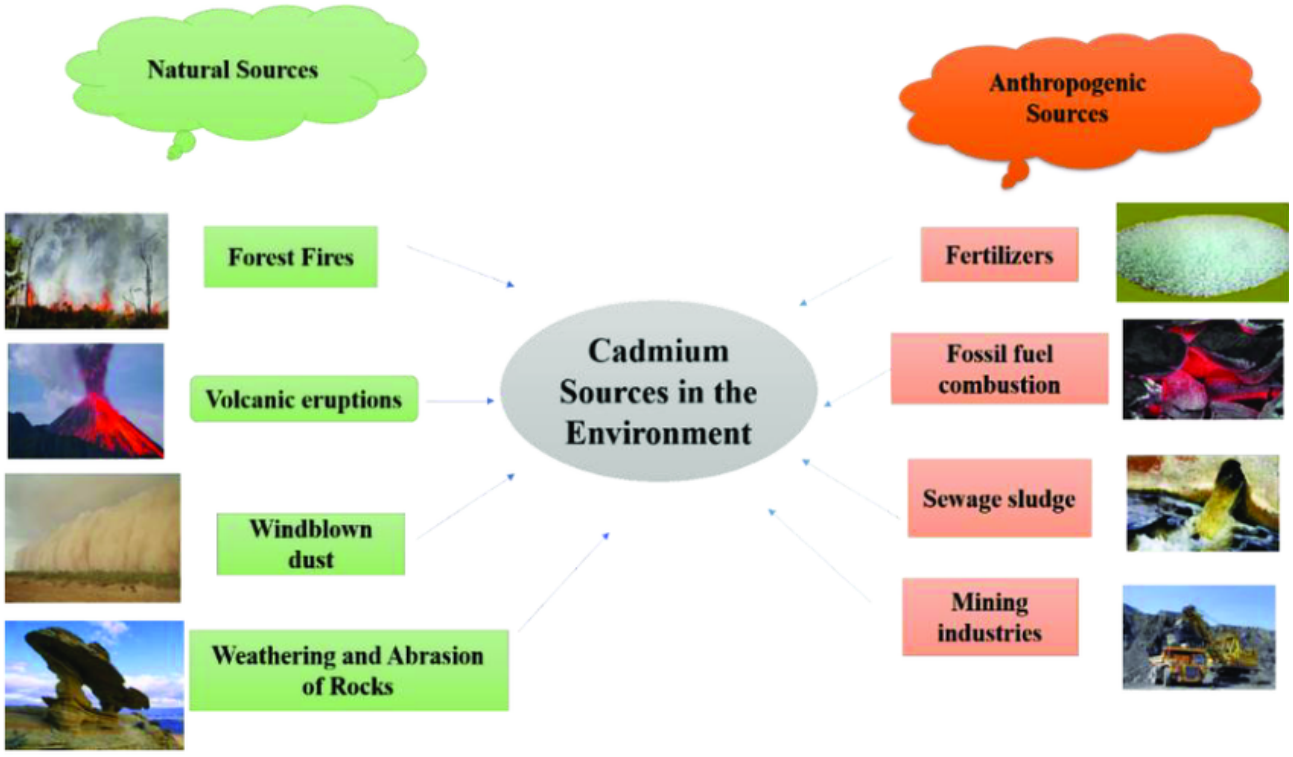

Figure 1. Sources of cadmium (Cd) contamination in the environment.

ization of the bone matrix. Lead exposure has been shown to impair the differentiation and functional activity of osteoblasts, leading to a reduction in their ability to form new bone. This inhibition is characterized by a decrease in the synthesis of critical bone matrix proteins, such as collagen and osteocalcin, which are necessary for the proper formation and maintenance of bone structure. The diminished osteoblast function caused by lead exposure disrupts the balance of bone remodeling by reducing bone formation rates, which in turn lowers bone density and increases the susceptibility to fractures.

2.2.2 Promotion of Osteoclast Differentiation

In addition to inhibiting osteoblast activity, lead exposure promotes bone resorption by enhancing the differentiation and activity of osteoclasts. This effect is primarily mediated through the dysregulation of the RANKL and OPG signaling pathway, which plays a pivotal role in osteoclastogenesis. Lead exposure increases the expression of RANKL, a key factor that stimulates osteoclast formation and activity, while simultaneously decreasing the levels of OPG, a natural inhibitor of RANKL. The resulting elevation in the RANKL/OPG ratio favors osteoclast differentiation, leading to enhanced bone resorption. The accelerated bone loss associated with increased osteoclast activity contributes to the reduction in bone mass and the weakening of the bone structure, thereby increasing the risk of osteoporosis [8].

2.2.3 Interference with Calcium and Vitamin D Metabolism

Calcium and vitamin D are essential for maintaining bone health, as they play crucial roles in bone mineralization and the regulation of bone turnover. Lead interferes with the metabolism of both calcium and vitamin D, further exacerbating its detrimental effects on bone. One of the mechanisms by which lead affects bone mineralization is by substituting for calcium in the hydroxyapatite crystals of the bone matrix, resulting in poor mineralization and weakened bone. Additionally, lead disrupts the hormonal regulation of calcium by affecting parathyroid hormone (PTH) levels, which can lead to altered calcium homeostasis. The impact of lead on vitamin D metabolism is also significant, as it impairs the renal conversion of vitamin D to its active form, 1,25-dihydroxyvitamin D, which is critical for calcium absorption in the gut and maintenance of adequate serum calcium levels.

The combined effects of lead on osteoblast impairment, osteoclast promotion, and interference with calcium and vitamin D metabolism create a scenario in which bone resorption is accelerated, and bone formation is inhibited. This disruption in bone remodeling results in decreased bone mineral density and structural integrity, contributing to the development of osteoporosis. Understanding these mechanisms is essential for developing therapeutic strategies to counteract the skeletal toxicity of lead and to reduce the risk of bone disease in populations exposed to this envi-

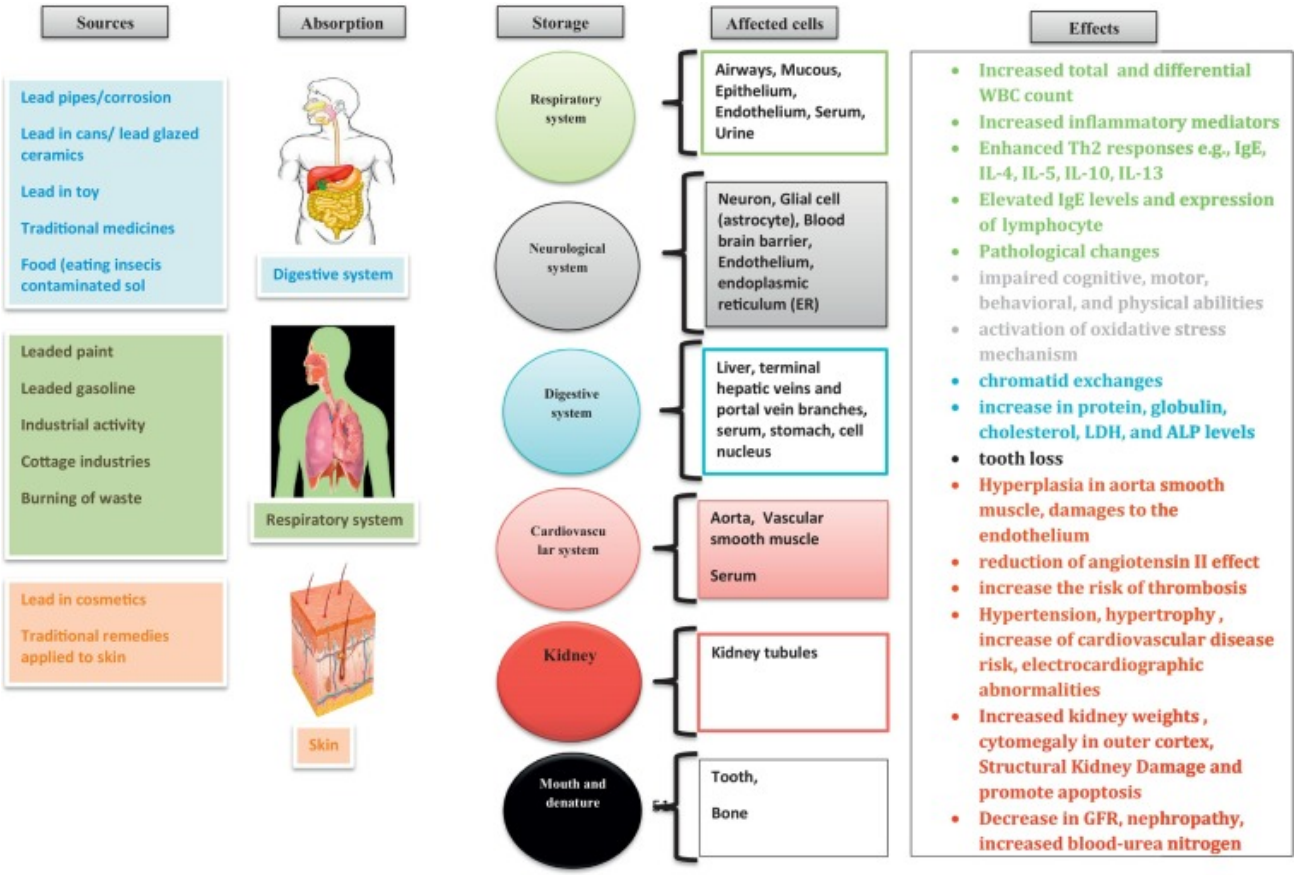

Figure 2. The effect of environmental lead exposure on human health

ronmental toxin.

2.3 Zinc

Zinc is a vital trace element that plays a fundamental role in bone health by contributing to the processes of bone formation and mineralization. It is a cofactor for numerous enzymes and transcription factors involved in bone metabolism, supporting the activity of osteoblasts while simultaneously inhibiting osteoclastogenesis under physiological conditions. However, while zinc is essential for maintaining bone integrity, excessive levels of zinc exposure can paradoxically have harmful effects on bone tissue. When present in high concentrations, zinc may contribute to skeletal pathology through mechanisms such as altering osteoblast and osteoclast function, inducing oxidative stress, and affecting the activity of matrix metalloproteinases (MMPs), all of which can disrupt the homeostatic balance of bone remodeling.

2.3.1 Role in Osteoblast and Osteoclast Function

At normal physiological concentrations, zinc serves to promote osteoblast activity, enhancing the synthesis of bone matrix proteins such as collagen and increasing alkaline phosphatase activity, which is crucial for bone mineralization. Additionally, zinc exerts an inhibitory effect on osteoclast differentiation by downregulating the RANKL pathway, thereby reducing bone resorption. However, when zinc concentrations become excessively elevated, these protective effects may be overridden. High levels of zinc have been associated with increased oxidative stress within bone cells, which can shift the balance toward osteoclast activation and bone resorption. The induction of oxidative stress by elevated zinc levels enhances osteoclastogenesis, thereby accelerating bone resorption and potentially leading to bone loss. This dual role of zinc, beneficial at physiological levels yet potentially harmful at higher concentrations, underscores the importance of maintaining zinc homeostasis for optimal bone health.

2.3.2 Oxidative Stress Induction

Oxidative stress is a significant factor in bone metabolism, influencing the balance between bone formation and resorption. Excess zinc can lead to the overproduction of reactive oxygen species (ROS), which in turn can damage cellular components, including proteins, lipids, and DNA. In the context of bone tissue, ROS disrupt the regulatory mechanisms that control the activity of osteoblasts and osteoclasts. High levels of ROS can inhibit osteoblast activity, reducing bone formation, while simultaneously promoting osteoclast differentiation and activity, thereby increasing

Table 3. Effects of Zinc on Bone Metabolism

Mechanism	Normal Physiological Role	Effect of Excessive Zinc Exposure
Osteoblast and Osteoclast Function	Promotes osteoblast activity, enhances synthesis of bone matrix proteins such as collagen, increases alkaline phosphatase activity, inhibits osteoclast differentiation by downregulating the *RANKL* pathway	High zinc levels increase oxidative stress, leading to enhanced osteoclastogenesis and bone resorption, potentially resulting in bone loss
Oxidative Stress	Maintains redox balance under normal conditions, preventing excessive reactive oxygen species (*ROS*) production	Excess zinc induces overproduction of *ROS*, which disrupts osteoblast and osteoclast activity, reducing bone formation and increasing bone resorption
Matrix Metalloproteinases (MMPs)	Serves as a cofactor for *MMP* activation, facilitating normal bone matrix turnover and remodeling	Elevated zinc levels can dysregulate *MMP* activity, leading to pathological degradation of bone matrix and compromised bone quality

bone resorption. The resultant imbalance between bone formation and bone resorption contributes to the deterioration of bone quality and increases the risk of osteoporosis. Understanding the role of zinc-induced oxidative stress in bone pathology provides insight into how excessive zinc exposure may lead to adverse skeletal outcomes [9].

2.3.3 Impact on Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) are a family of enzymes that play a key role in the turnover and degradation of the extracellular matrix components, including those found in bone tissue. Zinc is a critical cofactor for the activation of MMPs, and thus it can influence their activity. Under normal conditions, MMPs contribute to bone remodeling by facilitating the resorption of old or damaged bone matrix, allowing for the deposition of new bone tissue. However, dysregulation of MMP activity, potentially induced by excessive zinc levels, can lead to pathological degradation of bone matrix components. This aberrant increase in MMP activity may impair the structural integrity of bone and compromise bone quality, further contributing to bone fragility. The impact of zinc on MMP regulation adds another layer to its complex role in bone metabolism, highlighting the necessity of maintaining zinc levels within an optimal range to support healthy bone remodeling.

3 MECHANISMS OF HEAVY METAL-INDUCED OSTEOPOROSIS

The mechanisms underlying heavy metal-induced osteoporosis involve a combination of cellular and molecular processes that disrupt normal bone remodeling. These mechanisms include oxidative stress, disruption of calcium homeostasis, and alterations in signaling pathways that regulate bone turnover.

3.1 Oxidative Stress

Oxidative stress is a critical factor in the pathogenesis of osteoporosis, particularly in cases associated with exposure to heavy metals such as cadmium, lead, and zinc. This pathological condition arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize these harmful compounds through antioxidant defenses. ROS are chemically reactive molecules containing oxygen, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-). When present in excessive amounts, ROS can cause oxidative damage to cellular components such as lipids, proteins, and DNA. In the context of heavy metal toxicity, the generation of ROS is significantly increased, leading to a cascade of cellular events that adversely affect bone health.

The impact of oxidative stress on bone metabolism is substantial, as it influences both bone formation and bone resorption. Bone remodeling is a dynamic process that involves the continuous turnover of bone matrix, maintained by the coordinated activity of osteoblasts, which form new bone, and osteoclasts, which resorb old bone. Under normal physiological conditions, a balance exists between these two processes, ensuring skeletal integrity. However, oxidative stress disrupts this balance by promoting osteoclastogenesis and impairing osteoblast function. ROS serve as signaling molecules that can activate various intracellular pathways, with the nuclear factor-kappa B (NF-κB) signaling pathway being one of the most important. NF-κB is a transcription factor that plays a crucial role in regulating immune responses, inflammation, and cell survival. In the context of bone remodeling, NF-κB is a key regulator of osteoclast differentiation and activity.

Heavy metal-induced oxidative stress enhances the activation of NF-κB, which in turn promotes the expression of genes involved in osteoclastogenesis, such as receptor activator of nuclear factor-kappa B ligand (RANKL). The binding of RANKL to its receptor RANK on the surface

of osteoclast precursors triggers the differentiation of these cells into mature osteoclasts. The increased production of RANKL and activation of NF-κB due to oxidative stress result in an elevated rate of bone resorption. This shift towards enhanced bone degradation contributes to the net loss of bone mass, a hallmark of osteoporosis. In addition to promoting osteoclast activity, ROS can impair the function of osteoblasts, which are responsible for bone formation. Oxidative stress inhibits the activity of key enzymes involved in the synthesis of bone matrix proteins, such as alkaline phosphatase, and disrupts signaling pathways necessary for osteoblast differentiation. As a result, bone formation rates decrease, further exacerbating bone loss.

The specific effects of different heavy metals on ROS generation and bone metabolism vary, yet share common pathways that contribute to oxidative damage. Cadmium is particularly potent in inducing oxidative stress, as it can deplete intracellular levels of glutathione, a critical antioxidant, and inhibit the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase. This depletion of cellular antioxidant defenses leads to the accumulation of ROS, causing oxidative damage to bone cells and the bone matrix. Cadmium's ability to induce oxidative stress has been linked to increased lipid peroxidation and protein carbonylation in bone tissue, both of which are markers of oxidative damage. These changes impair the structural integrity of the bone and increase its susceptibility to fractures [10].

Lead exposure similarly contributes to oxidative stress in bone by disrupting the balance between pro-oxidant and antioxidant systems. Lead can replace essential divalent cations, such as calcium, in cellular processes, thereby interfering with the normal function of antioxidant enzymes. Furthermore, lead exposure is associated with increased production of pro-inflammatory cytokines, which can activate the NF-κB pathway and promote osteoclastogenesis. The combined effects of lead on oxidative stress and inflammation create a pathological environment that favors bone resorption over bone formation, leading to a decrease in bone mineral density (BMD).

Zinc, while an essential trace element for bone health at physiological levels, can exert toxic effects when present in excess. High concentrations of zinc have been shown to generate ROS through mechanisms that involve the disruption of mitochondrial function. The excessive production of ROS resulting from elevated zinc levels can activate the NF-κB pathway and promote osteoclast differentiation, thereby increasing bone resorption. Additionally, the induction of oxidative stress by high zinc levels may impair osteoblast function, reducing bone formation and contributing to bone fragility.

The detrimental effects of ROS on bone health are not limited to the promotion of osteoclast activity. ROS can also modulate the expression and activity of matrix metalloproteinases (MMPs), which are enzymes involved in the degradation of the extracellular matrix. The activation of MMPs in response to oxidative stress can lead to excessive breakdown of the bone matrix, further compromising bone quality. Additionally, oxidative damage to the bone matrix itself, through processes such as lipid peroxidation, can weaken the structural integrity of bone tissue, making it more prone to fractures.

The body possesses several antioxidant defense mechanisms that counteract the harmful effects of ROS, including enzymatic and non-enzymatic systems. Enzymatic antioxidants such as SOD, glutathione peroxidase (GPx), and catalase play essential roles in detoxifying ROS. However, heavy metal exposure can compromise these defense systems, either by directly inhibiting the activity of antioxidant enzymes or by depleting the levels of cofactors required for their function. For example, cadmium's interference with glutathione synthesis reduces the availability of this critical antioxidant, while lead exposure can inhibit the activity of SOD and GPx. This impairment of antioxidant defenses exacerbates the accumulation of ROS in the bone microenvironment, creating a vicious cycle of oxidative damage and bone loss.

In addition to enzymatic antioxidants, non-enzymatic antioxidants such as vitamins C and E, and small molecules like glutathione, also contribute to the neutralization of ROS. These antioxidants can scavenge free radicals and prevent the oxidative modification of cellular components. However, the efficacy of non-enzymatic antioxidants in protecting against heavy metal-induced oxidative stress is often limited by the overwhelming production of ROS in the presence of these toxicants.

The role of oxidative stress in heavy metal-induced bone disease underscores the importance of developing therapeutic strategies that target this pathological process. Antioxidants that enhance the activity of the body's natural defense mechanisms may offer potential for mitigating the skeletal toxicity associated with heavy metal exposure. For instance, compounds that boost glutathione synthesis or upregulate the expression of antioxidant enzymes could help restore the balance between pro-oxidant and antioxidant systems. Additionally, pharmacological agents that inhibit the activation of the NF-κB pathway may help reduce osteoclast activity and prevent bone resorption.

Understanding the mechanisms by which oxidative stress contributes to the pathogenesis of osteoporosis provides valuable insights into potential therapeutic interventions. As heavy metal pollution remains a significant public health concern, particularly in industrial and mining areas, addressing the oxidative stress component of metal toxicity is crucial for the prevention and treatment of bone disorders. Future research should focus on identifying effective antioxidant therapies and protective agents that can counteract the detrimental effects of heavy metals on bone health, as well as public health policies aimed at reducing environmental exposure to these toxicants.

Moreover, oxidative stress has deleterious effects on osteoblasts, the bone-forming cells. ROS can impair the function of osteoblasts by inhibiting the activity of crucial enzymes involved in the synthesis and mineralization of the bone matrix, such as alkaline phosphatase. This inhibition reduces bone formation rates, tipping the balance of bone remodeling towards resorption. In animal models such as *Peromyscus leucopus*, studies have demonstrated that exposure to heavy metals like cadmium, lead, and zinc significantly increases oxidative damage in bone tissue. Markers of oxidative stress, such as lipid peroxidation products and protein carbonylation, have been found to be elevated in the bone of these animals following heavy metal exposure, indicating that oxidative damage plays a direct role in the compromised bone quality observed [11].

The body's antioxidant defense mechanisms are crucial for counteracting the harmful effects of ROS. Key components of this defense system include enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which function to detoxify superoxide radicals and hydrogen peroxide, respectively. However, heavy metal exposure can impair these antioxidant systems, either by directly inhibiting the activity of SOD and GPx or by depleting cellular levels of essential cofactors required for their function. The resulting compromise in antioxidant defense leads to an accumulation of ROS within the bone microenvironment, further exacerbating oxidative damage to bone cells and matrix components.

The persistent presence of ROS and the diminished antioxidant capacity create a pro-inflammatory environment that contributes to bone loss. Inflammatory cytokines, which are upregulated in response to oxidative stress, can further stimulate osteoclast differentiation and activity while inhibiting osteoblast function, thereby perpetuating the cycle of bone degradation. The interplay between oxidative stress and inflammation in the bone microenvironment thus represents a significant mechanism by which heavy metal exposure promotes osteoporosis.

Understanding the role of oxidative stress in heavy metal-induced bone disease is essential for developing strategies aimed at mitigating its impact. Therapeutic approaches that target oxidative stress pathways, such as the use of antioxidants to enhance the body's defense mechanisms, may offer potential for preventing or slowing the progression of osteoporosis caused by heavy metal exposure. Additionally, reducing exposure to heavy metals through environmental regulations and public health interventions remains a critical component in the prevention of bone disorders associated with toxic metal exposure.

3.2 Disruption of Calcium Homeostasis

The regulation of calcium homeostasis is crucial for maintaining bone health, as calcium is a primary component of the bone matrix and is essential for various physiological functions, including nerve transmission, muscle contraction, and blood clotting. However, exposure to heavy metals such as cadmium and lead can significantly disrupt the normal metabolism of calcium, leading to adverse effects on skeletal health. These metals interfere with the absorption of calcium in the intestines and its reabsorption in the kidneys, resulting in alterations in serum calcium levels that can have profound implications for bone remodeling.

Cadmium disrupts calcium absorption by competing with calcium ions in the gastrointestinal tract, thereby reducing the amount of calcium available for incorporation into the bone. Additionally, cadmium's nephrotoxic effects impair renal reabsorption of calcium, increasing urinary calcium excretion and contributing to hypocalcemia. This reduction in circulating calcium levels triggers compensatory mechanisms aimed at restoring serum calcium homeostasis. One such mechanism is the release of parathyroid hormone (PTH), which is secreted by the parathyroid glands in response to low serum calcium levels. PTH acts to increase serum calcium concentrations by stimulating osteoclast activity, leading to the resorption of bone tissue and the mobilization of calcium into the bloodstream. While this compensatory process helps to maintain adequate levels of circulating calcium, it also accelerates bone loss, as the increased osteoclast-mediated resorption reduces bone mineral density.

Lead exposure similarly affects calcium metabolism by mimicking calcium ions and incorporating into the bone matrix in place of calcium. This substitution compromises the quality of the bone matrix, leading to poor mineralization and increased bone fragility. Moreover, lead disrupts the hormonal regulation of calcium by interfering with the activity of PTH and vitamin D, both of which are crucial for maintaining calcium balance. Lead has been shown to impair the renal conversion of vitamin D to its active form, calcitriol (1,25-dihydroxyvitamin D), which is necessary for the efficient absorption of dietary calcium in the intestines. The combined effects of impaired calcium absorption, increased urinary excretion, and altered hormonal regulation exacerbate the disruption of calcium homeostasis, thereby promoting bone resorption and contributing to the development of osteoporosis [12].

The chronic disruption of calcium homeostasis caused by heavy metal exposure creates a pathological cycle in which bone resorption is continually favored over bone formation. This imbalance in bone remodeling not only leads to a decrease in bone mass but also compromises the structural integrity of the skeleton, increasing the risk of fractures. Understanding the mechanisms through which heavy metals disrupt calcium metabolism is essential for developing therapeutic strategies that can mitigate their skeletal toxicity. Approaches that aim to improve calcium absorption, enhance the activity of the body's natural compensatory mechanisms, or chelate heavy metals to reduce their bioavailability may offer potential avenues for preventing bone loss associated with heavy metal exposure.

3.3 Alterations in Signaling Pathways

The homeostatic regulation of bone remodeling is governed by several critical signaling pathways that coordinate the activities of osteoblasts and osteoclasts, thereby maintaining a balance between bone formation and resorption. This balance is essential for preserving skeletal integrity and ensuring bone quality throughout life. However, exposure to heavy metals such as cadmium, lead, and zinc has been shown to disrupt these regulatory pathways, resulting in an imbalance that favors bone resorption over bone formation—a pathological state that is a hallmark of osteoporosis. The mechanisms by which heavy metals affect bone homeostasis are complex and multifaceted, involving alterations in key signaling pathways that regulate osteoblast and osteoclast function. Two of the most important pathways affected by heavy metal exposure are the RANK/RANKL/OPG system and the Wnt/β-catenin signaling pathway, both of which play pivotal roles in the regulation of bone remodeling.

The RANK/RANKL/OPG system is a central mediator of osteoclastogenesis, the process by which osteoclasts differentiate and become activated to resorb bone tissue. In this signaling pathway, receptor activator of nuclear factor-kappa B (RANK) is a receptor expressed on the surface of osteoclast precursors, while RANK ligand (RANKL) is expressed by osteoblasts and stromal cells. The binding of RANKL to RANK promotes the differentiation, activation, and survival of osteoclasts, leading to increased bone resorption. Osteoprotegerin (OPG) acts as a decoy receptor that binds to RANKL, preventing its interaction with RANK and thereby inhibiting osteoclastogenesis. Heavy metal exposure has been shown to disrupt this system by upregulating the expression of RANKL and downregulating the expression of OPG, resulting in an increased RANKL/OPG ratio. This imbalance enhances osteoclast activity, accelerates bone resorption, and contributes to the net loss of bone mass observed in osteoporosis. For instance, cadmium has been found to stimulate the expression of RANKL in bone tissue while simultaneously reducing OPG levels, thereby promoting osteoclast-mediated bone degradation [13].

In addition to its effects on the RANK/RANKL/OPG system, heavy metal exposure can also disrupt the Wnt/β-catenin signaling pathway, which is crucial for osteoblast differentiation and bone formation. The Wnt/β-catenin pathway is activated when Wnt proteins bind to cell surface receptors known as Frizzled and low-density lipoprotein receptor-related proteins (LRP5/6), leading to the stabilization and accumulation of β-catenin in the cytoplasm. Stabilized β-catenin then translocates into the nucleus, where it acts as a transcriptional coactivator for target genes involved in osteoblast proliferation, differentiation, and bone matrix synthesis. Disruption of this pathway by heavy metals can impair osteoblast function and reduce bone formation rates. For example, lead exposure has been shown to inhibit Wnt signaling by promoting the degradation of β-catenin, thereby suppressing osteoblast activity and contributing to decreased bone density.

The interplay between the RANK/RANKL/OPG and Wnt/β-catenin pathways is significant in the context of bone remodeling, as these pathways not only regulate the activities of osteoclasts and osteoblasts, respectively, but also interact with each other to maintain a balanced remodeling process. When the RANK/RANKL/OPG system is activated due to heavy metal exposure, the resultant increase in osteoclast activity can generate signals that inhibit the Wnt/β-catenin pathway, further reducing osteoblast-mediated bone formation. Conversely, disruptions in the Wnt/β-catenin pathway that impair osteoblast function can indirectly promote osteoclastogenesis by altering the expression of osteoprotegerin and other regulatory factors. This interconnected regulation makes the bone remodeling process highly susceptible to disturbances caused by toxicants that affect multiple signaling cascades.

Heavy metals such as cadmium, lead, and zinc also affect other signaling pathways linked to bone metabolism, including those involving oxidative stress and inflammation. These pathways can act in concert with the RANK/RANKL/OPG and Wnt/β-catenin systems to exacerbate bone resorption and inhibit bone formation. For example, oxidative stress resulting from heavy metal exposure can activate the nuclear factor-kappa B (NF-κB) pathway, which not only promotes inflammation but also enhances the expression of RANKL, thereby amplifying osteoclast activity. The cumulative effect of these disruptions is a shift in the bone remodeling balance toward resorption, leading to the progressive loss of bone mass and increased fracture risk characteristic of osteoporosis.

Understanding how heavy metals disrupt key signaling pathways in bone remodeling is essential for developing targeted therapies aimed at mitigating their skeletal toxicity. Interventions that inhibit the RANK/RANKL interaction or activate the Wnt/β-catenin pathway may offer potential therapeutic strategies to restore bone balance and improve bone health in individuals exposed to heavy metals. Additionally, research aimed at identifying agents that can modulate these signaling pathways, either by enhancing the expression of protective factors like OPG or by stabilizing β-catenin, could provide new avenues for the prevention and treatment of osteoporosis associated with environmental toxicants.

The RANK/RANKL/OPG system plays a central role in the regulation of osteoclastogenesis, the process by which osteoclasts differentiate and mature. Receptor activator of nuclear factor-kappa B (RANK) is a receptor on the surface of osteoclast precursors, while RANK ligand (RANKL), expressed by osteoblasts and stromal cells, binds to RANK to promote osteoclast differentiation and activation. Osteoprotegerin (OPG) is a decoy receptor that binds RANKL, thereby inhibiting its interaction with RANK and acting as a natural inhibitor of osteoclastogenesis. Heavy metals such

as cadmium and lead have been found to upregulate the expression of RANKL while downregulating OPG, resulting in an increased RANKL/OPG ratio. This shift in the balance of the RANK/RANKL/OPG system leads to enhanced osteoclast activity and bone resorption, accelerating bone loss and contributing to the pathogenesis of osteoporosis.

In addition to their effects on the RANK/RANKL/OPG system, heavy metals also influence the Wnt/β-catenin signaling pathway, which is critical for osteoblast differentiation and bone formation. The Wnt/β-catenin pathway is activated when Wnt proteins bind to specific cell surface receptors, such as Frizzled and low-density lipoprotein receptor-related proteins (LRP5/6). This binding initiates a cascade of intracellular events that lead to the stabilization and accumulation of β-catenin in the cytoplasm. The stabilized β-catenin then translocates to the nucleus, where it functions as a transcriptional coactivator, promoting the expression of target genes involved in osteoblast function, bone matrix synthesis, and mineralization. Through these mechanisms, the Wnt/β-catenin pathway plays a vital role in promoting bone formation and maintaining bone mass.

Heavy metal exposure has been shown to disrupt the Wnt/β-catenin pathway, leading to detrimental effects on osteoblast activity and bone health. For instance, cadmium exposure is known to downregulate key components of the Wnt/β-catenin signaling pathway, thereby inhibiting the accumulation of β-catenin in the cytoplasm and its subsequent translocation to the nucleus. This disruption impairs the transcription of osteoblast-specific genes, leading to decreased differentiation of osteoblasts, reduced bone formation rates, and impaired mineralization of the bone matrix. The downregulation of Wnt/β-catenin signaling by cadmium ultimately contributes to a reduction in bone density and an increased susceptibility to fractures.

Lead exposure similarly affects the Wnt/β-catenin pathway by promoting the degradation of β-catenin, thus preventing its stabilization and nuclear translocation. This effect inhibits the activation of osteogenic genes, leading to a decrease in osteoblast proliferation and function. The resulting impairment of bone formation, when combined with the increased osteoclast activity driven by changes in the RANK/RANKL/OPG system, further exacerbates the imbalance in bone remodeling caused by heavy metal toxicity. Such disruptions in the Wnt/β-catenin pathway compromise the bone's ability to repair and regenerate, thereby reducing bone quality and increasing the risk of osteoporosis [14].

The combined effects of altered RANK/RANKL/OPG signaling and impaired Wnt/β-catenin activity create a pathological environment in which bone resorption predominates over bone formation. This disruption in signaling cascades ultimately leads to an imbalance in bone remodeling, resulting in decreased bone mass, compromised bone quality, and an elevated risk of osteoporosis. The shift towards increased bone resorption and reduced bone formation not only weakens the structural integrity of the bone but also disrupts the microarchitectural organization, which is essential for bone strength.

Understanding how heavy metals influence these signaling pathways provides important insights into the molecular mechanisms underlying heavy metal-induced bone disease. The disruption of both the RANK/RANKL/OPG and Wnt/β-catenin pathways highlights the complexity of the processes involved in bone remodeling and the multifaceted nature of osteoporosis caused by environmental toxicants. Research into the molecular targets affected by heavy metals can help identify potential points of intervention, offering opportunities to develop therapeutic strategies aimed at restoring balance in bone remodeling.

Therapeutic approaches to mitigate the effects of heavy metal exposure on the skeletal system may involve targeting these disrupted signaling pathways. Modulating the RANK/RANKL/OPG ratio to inhibit osteoclast activity represents one such strategy, which could reduce excessive bone resorption and slow the progression of bone loss. Agents that block the interaction between RANKL and RANK, such as denosumab, have already been used in clinical settings to treat osteoporosis by inhibiting osteoclast-mediated bone resorption. Furthermore, enhancing Wnt/β-catenin signaling to promote osteoblast differentiation and function could help to restore bone formation rates and improve bone density. Potential therapeutic agents, such as Wnt pathway activators or β-catenin stabilizers, may offer novel ways to counteract the adverse effects of heavy metal exposure on bone health.

4 CONCLUSION

Exposure to heavy metals, particularly cadmium, lead, and zinc, exerts significant detrimental effects on bone health, contributing to the pathogenesis of osteoporosis. These metals induce bone loss through multifaceted mechanisms that disrupt the normal processes of bone remodeling. The pathways affected include the induction of oxidative stress, disruption of calcium homeostasis, and alterations in critical signaling pathways such as the RANK/RANKL/OPG system and the Wnt/β-catenin pathway. The combined impact of these disruptions leads to an imbalance between bone resorption and formation, resulting in decreased bone density, compromised bone quality, and an elevated risk of fractures.

The induction of oxidative stress is one of the primary mechanisms by which heavy metals cause bone damage. Oxidative stress arises from an excess of reactive oxygen species (ROS), which overwhelm the body's antioxidant defenses. These ROS can damage cellular components and disrupt the signaling pathways that regulate osteoclast and osteoblast activity. Specifically, oxidative stress activates the nuclear factor-kappa B (NF-κB) pathway, which promotes osteoclastogenesis and increases bone resorption. At the same time, ROS impair osteoblast function, leading to

reduced bone formation. The cumulative effect of increased bone resorption and diminished bone formation accelerates the loss of bone mass associated with osteoporosis.

In addition to oxidative stress, heavy metals interfere with calcium homeostasis, which is crucial for maintaining bone health. Cadmium and lead can disrupt calcium absorption in the intestines and its reabsorption in the kidneys, leading to alterations in serum calcium levels. This disruption triggers compensatory mechanisms such as the release of parathyroid hormone (PTH), which stimulates osteoclast activity to restore calcium levels by resorbing bone. The resulting increase in bone resorption further exacerbates bone loss and compromises skeletal integrity.

Heavy metals also disrupt key signaling pathways involved in bone remodeling, such as the RANK/RANKL/ -OPG system and the Wnt/β-catenin pathway. The RANK/ -RANKL/ -OPG system is central to regulating osteoclast differentiation and activity, with an increased RANKL/OPG ratio favoring bone resorption. Exposure to heavy metals has been shown to upregulate RANKL expression and downregulate OPG, thus promoting osteoclastogenesis and accelerating bone degradation. Concurrently, heavy metals impair the Wnt/β-catenin signaling pathway, which is essential for osteoblast differentiation and bone formation. The inhibition of Wnt/β-catenin signaling reduces osteoblast activity and impairs bone mineralization, contributing to decreased bone density and quality [15].

The insights gained from this study provide a deeper understanding of the specific ways in which heavy metal exposure affects bone turnover. By elucidating the molecular mechanisms through which cadmium, lead, and zinc disrupt bone remodeling, the findings highlight the importance of addressing environmental toxicants as part of strategies to prevent and treat osteoporosis. The study underscores the need for public health measures to reduce exposure to heavy metals and for the development of therapeutic approaches that target the underlying mechanisms of metal-induced bone disease.

Future research should focus on identifying agents that can mitigate the effects of heavy metal exposure on bone health. Potential strategies may involve antioxidants to combat oxidative stress, agents that modulate the RANK/ RANKL/ OPG pathway to reduce osteoclast activity, and drugs that enhance Wnt/β-catenin signaling to promote osteoblast function. Additionally, public health policies aimed at reducing environmental contamination and limiting human exposure to toxic metals will be crucial in the broader effort to prevent bone disorders linked to heavy metal toxicity. Overall, a comprehensive approach that integrates prevention, treatment, and policy initiatives will be necessary to protect skeletal health in populations at risk of heavy metal exposure.

The findings underscore the need for future research aimed at developing effective interventions to counteract the skeletal toxicity associated with heavy metal exposure. Such research should explore pharmacological agents that target oxidative stress, modulate disrupted signaling pathways, and restore calcium homeostasis. Additionally, protective agents such as dietary supplements or pharmacological inhibitors that can enhance the body's natural defense mechanisms against metal-induced damage may offer therapeutic potential. Given the pervasive nature of heavy metal contamination in the environment, there is also a critical need for public health policies that reduce human exposure to these toxicants and promote the resilience of bone tissue in populations at risk. The results of this investigation lay the groundwork for further studies on therapeutic approaches that can mitigate the impact of heavy metal toxicity on bone health. Research efforts should not only focus on treatment but also on preventative strategies, including the identification of dietary or lifestyle factors that can minimize heavy metal absorption or facilitate its excretion. Overall, a multidisciplinary approach that integrates toxicology, endocrinology, and environmental science is essential to advancing our understanding of heavy metal-induced osteoporosis and to developing comprehensive strategies for maintaining skeletal health in polluted environments.

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