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Research Progress on the Impact Mechanism of Gut Microbiota on Osteoporosis and the Application of Traditional Chinese Medicine

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Abstract: ***Osteoporosis (OP) is a metabolic disease that causes bone loss and microstructure degradation. The gut microbiota is the biggest microbial population in the human body and is considered the second largest human genome. Its encoded genome quantity is more than 150 times that of the human genome. With the further development of gut microbiology and in-depth research on gut microbiota (GM), we have found that changes in gut microbiota are closely related to changes in bone mass and microstructure. In this review, we elucidate several potential mechanisms of the microbiota gut bone axis, including endocrine hormones, immune regulation, gut microbiota metabolites, and gut permeability. A brief summary is presented on the beneficial therapeutic benefits of probiotics, prebiotics, and traditional Chinese medicine in correcting gut microbiota imbalance, which improves bone metabolism and promotes osteoporosis. Propose novel research ideas for osteoporosis prevention and treatment objectives, tactics, and mechanisms that might become new targets for preventive or therapy.***

Keywords: Acute severe pancreatitis; Etiology and risk factors; Diagnosis and treatment; Complications; Prognosis and rehabilitation.

1. Introduction

Osteoporosis (OP) is a metabolic disease that causes reduced bone mass, bone microstructure breakdown, increased bone fragility, and an increased risk of fractures. The frequency of osteoporosis has increased in recent years, as the world population has aged. The fractures caused by osteoporosis have brought heavy economic burden to society and families. Osteoporosis fractures mainly include spinal fractures, upper limb fractures, and hip fractures, which are closely related to a decrease in bone density (BMD). As a result, osteoporosis treatment and prevention are significant public health concerns. The gut microbiota refers to the trillion dollar microbial community that inhabits the gastrointestinal tract for a long time. It is the largest microbial community in the human body and is usually distributed on the surface of the host's intestinal mucosa. [1] composition of gut microbiota is influenced by age, geographical location, dietary habits, and lifestyle. The gut microbiota consists mostly of five bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Verrucomycota. In the human gut

microbiota, especially Bifidobacteria in the Actinobacteria phylum, they dominate the infant gut microbiota, while Firmicutes and Bacteroidetes account for about 90% of the total adult gut microbiota [2].

Bone microbiology is an interdisciplinary discipline that combines gut microbiology with skeletal biology. The gut microbiota is a crucial microbiota in the human body, closely linked to human health, and has been proposed as a potential treatment or prevention measure for many health diseases [3]. The "microbiota gut bone" axis suggests a close correlation between gut microbiota and osteoporosis. After research, it has been found that the gut microbiota inhibits osteoclast activity, reduces bone loss, and avoids osteoporosis by regulating hormone and immune responses, gut metabolites, intestinal mucosal permeability, and other mechanisms.

2. The mechanism of gut microbiota regulating osteoporosis

2.1 Bone Immunology: The Effects of GM on the Immune System

Aaron and Choi coined the phrase "bone immunology" in 2000, describing an interdisciplinary study topic that combines immunology and bone biology. In recent years, research has revealed that the gut microbiota and immune system play critical roles in bone homeostasis. The gut microbiota can modulate the immune system through a variety of routes, influencing bone metabolism. The gut microbiota activates immune cells at the intestinal endothelium barrier with metabolites, activating the immune system. The immune system and gut microbiota interact bidirectionally, with the immune system regulating gut microbial survival. The microbiome has an impact on how the immune system develops and functions.

T cells make up around 5% of bone marrow cells in both the stroma and the parenchyma. T cells can develop into CD4+ and CD8+ T cells, with regulatory T cells (Treg) and helper T cell-17 (Th17) cells being subsets of CD4+ cells that influence bone dynamic balance. Treg cells have immunosuppressive properties and are regarded as bone health defenders. Treg cells block osteoclast development, slow bone resorption, and prevent bone loss [4]. Th17 cells, on the other hand, can stimulate the immune system and serve as an effective target for bone degradation. Th17 cells have high amounts of pro-inflammatory factors (IL-17, RANKL, TNF-α) and low levels of anti-inflammatory factors (IFN-γ and IL-10), which can accelerate osteoclast production, enhance bone resorption, and loss [5]. SCFAs (short chain fatty acids), especially butyrate, can induce Treg cell differentiation, inhibit Th17 cell differentiation, regulate Treg/Th17 cell balance or related cytokines that affect bone remodeling [6], Once again demonstrating that gut bacteria may influence bone metabolism by modulating the immune system. Lactobacillus rhamnosus improves bone health in postmenopausal osteoporosis mice by inhibiting osteoclast Th17 cells and promoting anti osteoclast Treg cells [7]. In summary, gut microbiota can regulate Treg/Th17 cell balance, reduce bone loss, and avoid postmenopausal osteoporosis.

2.2 Endocrine system

In addition to the immune system, endocrine hormones are important in maintaining bone homeostasis. The hypothalamic pituitary adrenal (HPA) axis is the body's primary endocrine system, and it plays an important role in stress reactions. Several studies in recent years have found a tight relationship between the GM and HPA axes. Huo et al. discovered in a sterile mouse (GF) experimental paradigm that mice can demonstrate high HPA axis activation under mild stress conditions. However, after supplementing with an appropriate amount of colon bacteria, the HPA axis can return to normal [8].

SCFAs can participate in bone metabolism by affecting hormone levels such as parathyroid hormone (PTH), insulin-like growth factor-1 (IGF-1), and serotonin (5-HT). Parathyroid hormone (PTH) is a calcification hormone released by the parathyroid gland that stimulates bone production or absorption and plays a crucial role in bone remodeling [9]. PTH has a bidirectional influence on bone mass, with the primary difference being whether the target cells are exposed to PTH constantly or intermittently. Continuous exposure to PTH (cPTH) can increase bone resorption and cause bone loss[10]; On the other hand, intermittent exposure to parathyroid hormone (iPTH) stimulates the production of osteoblasts, coordinates the recruitment of osteoblasts, and promotes bone formation, playing a role in bone protection [11]. PTH induces differentiation of CD4+T cells into Treg cells by binding to PTH receptors (PTH1R) on CD4+T cells. On the contrary, Treg cells stimulate conventional CD8+T cells to produce the osteogenic Wnt ligand Wnt10b, which increases the activity of osteoblasts on the bone surface and stimulates bone synthesis metabolism by activating the Wnt signaling pathway in stromal cells and osteoblasts [12].

One neurotransmitter with significant and intricate physiological effects on bone remodeling is 5-hydroxytryptamine (5-HT). 5-HT is mostly biosynthesised in the human gastrointestinal tract, and the gut microbiota has a significant impact on blood 5-HT concentrations [13]. Gut microbiota appears to have an impact on serotonin synthesis and release, as evidenced by the considerably greater blood levels of serotonin in conventionally fed mice compared to GF animals. Osteoblasts and bone cells contain 5-hydroxytryptamine receptors, and the distribution of serotonin production in the body affects bone mass differently. According to earlier research, 5-HT derived from the brain favorably controls bone metabolism, but 5-HT coming from the gut decreases bone formation and causes negative feedback regulation on bone [14]. Inhibiting intestinal serotonin synthesis may prevent bone loss caused by ovarian resection by increasing the number of osteoblasts and bone formation in mice [15]. Thus, by controlling serotonin levels, the gut flora in postmenopausal osteoporosis patients can impact bone mass.

Insulin like growth factor-1 (IGF-1) is another significant way that the gut microbiome influences bone homeostasis. IGF-1 controls bone mass by either autocrine or paracrine processes and is mostly produced in the liver. IGF-1 is a growth factor that controls the activity of osteoblasts and osteoclasts in addition to encouraging the development of cartilage and long bones. Type I collagen, alkaline phosphatase, and osteocalcin are secreted in response to the activation of osteoblasts by IGF-1 receptors, which also causes the production of Runx2 in osteoblasts [16]. By stimulating the mTOR signaling cascade, attaching directly to IGF-1 receptors on osteoblasts, and encouraging osteoblast production and differentiation, IGF-1 can facilitate the conversion of mesenchymal stem cells into osteoblasts. In Yan's work, serum IGF-1 levels significantly increased in GF mice colonized by gut microbiota at 1 and 8 months post-colonization, suggesting a direct relationship between gut microbiota and IGF-1 secretion [17]. As a result, the metabolism of IGF-1 to control bone mass is mediated by the gut microbiota and its metabolites. Future studies should investigate the mechanism by which IGF-1 influences the gut microbiota's role in bone metabolism.

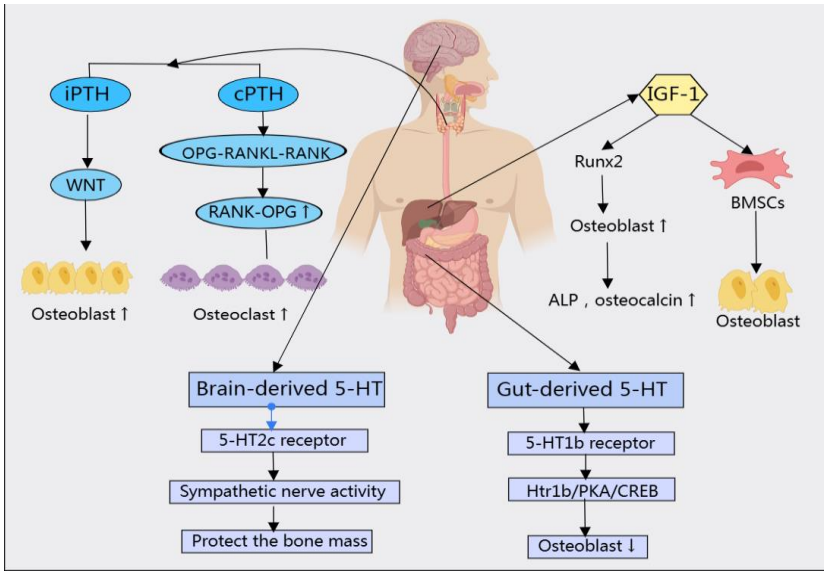

Figure 1: (1) Brain-derived 5-HT preserved bone mass, but gut-derived 5-HT inhibited the differentiation of osteoblasts via the Htr1b/PKA/CREB signaling pathway to alter bone metabolism. (2) iPTH and cPTH produce two totally different outcomes for bone. cPTH enhances RANKL/OPG signaling to increase the differentiation of osteoclasts. However, iPTH promotes bone formation by activating Wnt signaling. (3) IGF-1 promotes bone formation by inducing the expression of Runx2 and promoting the transformation of BMSCs into osteoblasts.

2.3 Intestinal metabolites

Several studies' findings have demonstrated the involvement of several intestinal metabolites in the control of bone metabolism, including short chain fatty acids, vitamins, serotonin, polyamines, trimethylamine oxide, tryptophan metabolites, etc. The main source of short chain fatty acids (SCFAs) is amino acids or carbohydrates produced by bacterial fermentation in the intestine. The main components include butyrate, propionate, and acetate, and they are one of the most important metabolites of gut microbiota [18]. Through a variety of mechanisms, such as reducing inflammatory response, enhancing intestinal calcium absorption, encouraging osteoblast differentiation via regulatory T cells, and directly preventing osteoclast differentiation, SCFAs can influence bone metabolism and take part in bone remodeling[19]. Because SCFAs modulate the Wnt and osteoprotegerin (OPG) signaling pathways, they have a significant impact on the development and mineralization of bone. In addition to directly regulating the differentiation of osteoclasts and osteoblasts, SCFAs can achieve anti osteoporosis effects by affecting mineral absorption. Experimental studies have shown that SCFAs can directly promote the absorption of mineral ions in intestinal epithelial cells by regulating the electrolyte exchange between mineral ions and hydrogen ions [20]. SCFAs can also lower the pH value in the intestine, promote the transport of calcium ions in the colon segment of rats, and increase calcium absorption. In addition, gut microbiota plays an important role in the metabolism of vitamin B, vitamin D, and vitamin K. Therefore, SCFAs have an impact on bone metabolism through a number of mechanisms, such as blocking the differentiation of osteoclasts, stimulating the growth of osteoblasts, encouraging the absorption of calcium, improving the function of the intestinal barrier, and eventually acting as a bone-protector.

Short chain fatty acids (SCFAs) are not the only gut microbiota metabolites that are crucial in preventing osteoporosis and lowering bone loss. Polyamines, trimethylamine oxide, and tryptophan are just a few examples. Small fatty amines called polyamines are essential for proper cellular operation. Recent studies have discovered that gut bacteria can stimulate the synthesis of exogenous polyamines in

addition to endogenous polyamine metabolism. By enhancing the strength and microstructure of the bone, polyamines can shield mice from ovx-induced bone loss[21]. Research has shown that the concentration of serum trimethylamine oxide (TMAO) in OP patients is significantly higher than that in healthy individuals, and TMAO activates NF by- κB signaling pathway induces adipogenesis in bone marrow mesenchymal stem cells, slows down bone formation, and also increases the release of reactive oxygen species and IL-1 β, IL-6, TNF-α The production of pro-inflammatory cytokines [22]. Tryptophan metabolites, especially canine uric acid (KYN) and serotonin, also affect normal bone metabolism. KYN has the ability to influence the process by which bone marrow mesenchymal stem cells proliferate into osteoblasts. It can also prevent osteoblast differentiation and rank-induced osteoclast formation, which can result in the rupture of bone structure [23]. The basic principle for preventing and treating OP remains boosting the activity of OB and inhibiting the activation of OC, even if these intestinal metabolites may contribute to the regulation of bone metabolism in distinct ways. In conclusion, we think that gut metabolites act as one of the bridges, contributing significantly to the equilibrium between bone synthesis and absorption, preserving bone mass, averting osteoporosis, and more.

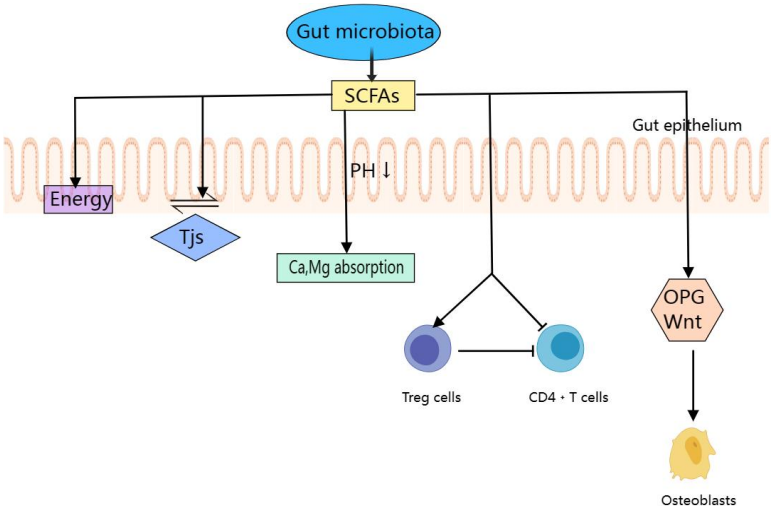

Figure 2: Effects of short-chain fatty acids (SCFAs) on bone metabolism. SCFAs can affect bone homeostasis via: (1) providing energy to intestinal epithelial cells; (2) Promote intestinal barrier integrity; (3) Enhance the absorption of Ca2+and Mg2+; (4) regulating the inflammatory response by triggering Treg cells differentiation while inhibiting CD4+ T cells; (5) Directly promote the activation of osteoblasts.

2.4 Intestinal mucosal barrier

The gut microbiota, external mucus, epithelium, and lamina propria make up the typical intestinal barrier. The intestinal mucosal barrier functions as a vital defense against pathogens in living things in addition to influencing nutrition absorption. In the event that inflammatory factors are triggered, the intestinal mucosal barrier's integrity is compromised, permeability increases, and the body is easily affected by intestinal microorganisms or other particles, thereby exacerbating inflammation, resulting in intestinal leakage. The intestinal mucosal barrier is composed of just one layer of continuously arranged intestinal epithelial cells, which are tightly connected and sealed together. Three proteins combine to form protein complexes known as tight junction proteins, which are essential for the permeability of extracellular solutes. The expression and distribution of tight junction proteins can be changed by the gut microbiota, which changes the intestinal barrier's permeability [24]. Damage to the intestinal barrier allows microorganisms to transfer from the intestinal cavity to the subcutaneous structure, triggering subsequent inflammatory reactions.

The decrease in estrogen levels increases intestinal mucosal permeability, causing the intestinal epithelium to produce tight junction proteins abnormally. Intestinal T cell translocation to the bone marrow is facilitated by OVX, and OVX-induced bone loss can be avoided by obstructing the passage of Th17 and TNF from the colon to the bone marrow [25]. Puerarin also has anti-osteoporosis benefits by restoring intestinal mucosal integrity and controlling levels of saturated fatty acids (SCFAs), which in turn controls the disturbance of gut microbiota caused by ovarian cancer (OVX) in rats, according to research by Li et al. [26].Based on the gut-bone axis, the potential mechanisms of gut microbiota transplantation (FMT) include restoring the normal gut microbiota, enhancing intestinal permeability, regulating immune response, and helping to regulate bone metabolism by mending the intestinal mucosal barrier, restoring intestinal metabolite imbalance, and treating gut microbiota problems [27]. Meanwhile, Zhang et al. showed that FMT improves bone loss in OVX-induced osteoporotic rats and has a beneficial function in gut microbiota remodeling [28]. To be more precise, FMT reduces bone loss in mice with OVX-induced osteoporosis by balancing the production of osteoclasts and bone formation, improving intestinal permeability, correcting imbalances in the gut microbiota, raising levels of SCFAs, and preventing the release of pro-inflammatory cytokines. It is important to note that these trials offer concrete proof that FMT can increase bone density and stave off osteoporosis. Therefore, improving the composition of gut microbiota and protecting intestinal barrier function will become new targets for the treatment of osteoporosis.

3. The application of traditional Chinese medicine in osteoporosis

Right now, dinozumab, terlipide, selective estrogen receptor modulators, and bisphosphonates are the major medications licensed to treat osteoporosis; each of these medications has potential negative effects. Owing to the shortcomings of conventional therapy, osteoporosis treatment with traditional Chinese medicine is now an option. According to recent research, people with osteoporosis may experience pharmacological benefits from traditional Chinese medicine that are mediated through their gut microbial species.

3.1 Single Traditional Chinese Medicine

Polyphenolic substances such lignans, phenolic acids, and flavonoids are among the bioactive elements found in the leaves and bark of Eucommia ulmoides. In OVX rats, the bark extract of Eucommia ulmoides prevents bone loss brought on by an estrogen shortage, mainly by regulating the activity of osteoblasts and osteoclasts [29]. Eucommia ulmoides leaf extract (EUL) prevents osteoporosis by altering the composition of gut microbiota in aging accelerated P6 mice. The water extract of Eucommia ulmoides leaves greatly reduced the abundance of Bacteroidetes and greatly enhanced the quantity of Firmicutes [30]. Ligustrum lucidum (FLL) is a plant of the olive family, and the dry and mature fruit of Ligustrum lucidum. Currently, more and more people are using it as a substitute drug for the prevention and treatment of osteoporosis [31]. By decreasing calcium loss and increasing calcium transport that is dependent on vitamin D, FLL therapy can improve osteoblast development and bone function. In the meantime, FLL may suppress NF via κ B-p65 activation and the production of CatK, which prevent the development of osteoclasts [32]. The primary active component of dried kudzu is called pururarin, and because it functions as a plant estrogen, it is frequently used to treat postmenopausal osteoporosis. Puerarin has a relatively poor bioavailability and may work against osteoporosis by altering the gut microbiome. [33]. After 14 weeks of oral treatment with puerarin, OVX rats repaired intestinal mucosal integrity, inhibited bone loss, and improved the fine structure of bone trabeculae by regulating the release of SCFAs from gut microbiota [26].

3.2 Common traditional Chinese patent medicines and simple preparations

Jiangu granules reduced the proportion of Firmicutes/Bacteroidetes at the phylum level and restored the structure of gut microbiota to reduce bone loss. Jiangu Granules can induce IL-4, IL-10, and TGF secretion by Treg cells in OVX rats- β The levels of IL-6, IL-17, and TNF secreted by Th17 cells increase to varying degrees- α Content decreases. Therefore, Jiangu granules may exert anti osteoporosis effects by regulating Treg/Th17 cell balance and its cytokines [34]. Xianling Bone Protection Capsule (XLGB) is a famous traditional Chinese medicine formula widely used in the treatment of osteoporosis. The gut microbiota of OVX mice treated with XLGB for three months can also change it. Disorders involving bile acid metabolism and lipid metabolism may be connected to the rise in the Firmicutes to Bacteroidetes ratio. Firmicutes to Bacteroidetes ratio was greatly lowered by XLGB therapy [35]. Studies have shown that XLGB can help with osteoporosis therapy by controlling the metabolism of lipids and bile acids. In conclusion, the richness of gut bacteria, preservation of intestinal mucosa integrity, inhibition of osteoclast production, and control over the synthesis of short chain fatty acids in the intestine are all ways that traditional Chinese medicine might prevent bone loss. It is crucial for both osteoporosis therapy and prevention.

4. Conclusion

With the aging of the global population, the number of patients with osteoporosis has significantly increased, but currently, the treatment of osteoporosis has not achieved the expected results. Luckily, a novel approach to osteoporosis therapy and prevention has been suggested: the "microbiota gut bone" axis. By controlling hormone production, modulating intestinal metabolites, immunological response, and intestinal mucosal barrier maintenance, the gut microbiota plays a role in the etiology of OP. The "microbiota gut bone" axis, one of the key regulatory elements of bone homeostasis, is intimately associated with osteoporosis and may be a target for osteoporosis prevention and treatment. Concurrently, fresh concepts have been put up for the formulation of goals, tactics, and procedures for OP prevention and control, as well as new avenues for OP research. Furthermore, even while OP research has focused on a variety of issues, such as molecular level, drug discovery, and clinical research in recent years, there is still a lot of work to be further explored on the profound impact of gut microbiota on OP. There are still significant challenges in the study of the relationship between gut microbiota and bones. In the past few years, there have been countless connections between gut microbiota and bones, but most of them have remained in animal experiments and require prospective clinical trials to determine the efficacy of prebiotics and probiotics in the prevention and treatment of osteoporosis.

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