INTRODUCTION

Osteoporosis (OP) is a systemic metabolic disease characterized by low bone mass and microstructural destruction of bone tissue, which leads to an increased bone fragility and easy fracture. The diagnosis of osteoporosis has always been concerned by the medical community. Diagnostic criteria recommended by WHO depends on the lumbar 1~4 and femoral neck measured by dual-energy X-ray bone densitometer. It is common that the value of bone density is less than 1 standard deviation (SD) in healthy adults of the same sex and race. The drop between −1 and −2.5 SD was identified to be low bone mass. Osteoporosis occurs when the reduction was equal to or greater than −2.5 SD. The incidence of osteoporosis is increasing, which has received more and more attention from the scientific researchers. Data from the National Osteoporosis Foundation show that osteoporosis affects 10.2 million Americans over the age of 50, while 43.4 million Americans have low bone mass (Looker, Borrud, Dawson-Hughes, Shepherd, & Wright, 2012). Moreover, the incidence of osteoporosis-related pathological fractures was far more than strokes, heart attacks, and breast cancer each year, which increased $19B fiscal burden of the state (Burge et al., 2007). It is well known that bone mass increases from birth and peaks in adulthood. The maintenance of bone in mid-to-late adulthood follows different age, sex, and race related patterns, which are greatly affected by lifestyle factors (Weaver et al., 2016). The absorption of calcium and vitamin D is particularly important for the maintenance of healthy bone, and other nutrients have been gradually discovered. For example, probiotics can reduce gut pH and improve calcium absorption (Wallace, Marzorati, Spence, Weaver, & Williamson, 2017) (Figure 1). During growth, fructose oligosaccharide, galactose, soluble corn fiber (SCF), and other probiotics can increase calcium uptake in humans. The pathogenesis of osteoporosis is complex.
Bone mass density is determined by bone absorption and bone formation. During a person’s life, bone remodeling continues, among which there are many influencing factors. When bone formation is dominant, it is characterized by anabolism, otherwise it’s catabolism. Hormonal environment, immune system, and metabolic pathways can affect this balance, and the gut microbiota could affect these pathways (Charles, Ermann, & Aliprantis, 2015).

Trillions of microbes live in the gut, evolving with their hosts together and forming mutually beneficial relationships (Backhed et al., 2012). These symbiotic microbes can be seen as a multicellular organ that interacts and influences host in a variety of ways (Clarke et al., 2014; Yano et al., 2015). Many studies have elucidated the role of intestinal microbiota in shaping the host immune system (Hooper, Dan, & Macpherson, 2012) as well as in host metabolism (Nieuwdorp, Gilijamse, Pai, & Kaplan, 2014). The composition of intestinal microbiota varies from individual to individual, while the involved functional genes are similar, which indicates that intestinal microbiota is an indivisible community to maintain the homeostasis of host (Methé et al., 2012). Probiotics are the active microorganisms that are beneficial to the host. Compared with ovariectomized mice without probiotic intervention, the bone mass of cortical bone was significantly increased in estrogen-deficient mouse model which was fed with Lactobacillus reuteri. Probiotics can inhibit the activity of osteoclasts and reduce the expression level of inflammatory factors. Besides, it can promote the absorption of bone calcium and significantly increase the expression of osteogenic markers (Resta-Lenert & Barrett, 2006). The natural prebiotics are mainly high fiber foods, including vegetables, fruits, and grains. Fructo-oligosaccharides, inulin-type prebiotics, can increase the amount of “beneficial” bacteria in the gut and promote the release of organic acids by stimulating the activity of bacteria, thereby lowering the pH of the intestines. Hence, prebiotics can promote the absorption of minerals and increase bone mineralization (María Isabel, Alicia, & López, 2014).

Taken together, intestinal microbiota may play a role in bone metabolism via influencing the host metabolism, immunity, and endocrine environment. Here, we searched relevant literature on Pubmed and then reviewed the effect of intestinal microbiota on bone metabolism through these three aspects.

2 | EFFECTS OF GUT MICROBIOTA ON HOST METABOLISM AND BONE HOMEOSTASIS

Diet can change the species of intestinal microbiota. High-fiber diet and fructo-oligosaccharides can increase the number of Bifidobacteria species (David, Maurice, & Carmody, 2014). Accumulating evidence suggests that probiotics can affect host metabolism, which may protect gut epithelial cell and maintain the integrity of mucous layer. By contrasting germ-free (GF) mice with conventionally fed mice, one previous study proved that intestinal microbiota impacts the host metabolism to influence bone density. GF mice obtained a 50% increase in femur trabecular bone volume and cortical bone (Sjogren et al., 2012). The intestinal microbiota can affect the host metabolism through a variety of ways to influence bone turnover. Here, we analyze several aspects of this field.
2.1 | Lipopolysaccharide (LPS)

In cross-sectional studies, high systemic LPS and LPS binding proteins were associated with low level of chronic inflammation leading to type 2 diabetes mellitus (T2DM), metabolic syndrome, and obesity (Cani et al., 2007, 2007; Creely et al., 2007; Jayashree et al., 2014; Sun et al., 2010). The bacterial cell wall composition of LPS and endotoxin is found mainly in gram-negative bacteria, which stimulate inflammation by activating transformed growth factor (TGF) and toll-like receptors 4 (Manco, Putignani, & Bottazzo, 2010). Characteristics of Dysbacteriosis in diabetes are reducing gram-positive bacteria which lacks LPS. Increasing gram-negative opportunistic pathogens, such as bacteroidetes and proteus species containing LPS are other characteristics in diabetes (Qin et al., 2012). Intestinal microbiota regulated cells permeability by sustaining the healthy and tight junctions of intestinal cells, and maintaining protective slime layer. Disorders of intestinal microbiota can increase the permeability of intestinal cell, which caused more LPS into the system of circulation, leading to metabolic dysfunction and inflammation (Horton, Wright, Smith, Hinton, & Robertson, 2013). There are many data in animal studies in this field, (Brun et al., 2007; Cani et al., 2007; Ghoshal, Witta, Zhong, De, & Eckhardt, 2009) more studies are needed in human. LPS also plays an important role in bone metabolism. Three doses of LPS time-release pellet were implanted in 3-month-old rats to mimic a vivo model of chronic inflammation.

In both LPS-treated groups, bone loss occurred in their femur, suggesting LPS may reduce bone mineral density. In the high-dose LPS group, microcomputed tomography indicated that trabecular bone volume of the proximal tibial metaphysis tended to be decreased. Furthermore, upregulation of the inflammatory mediators, interleukin (IL)-1, cyclooxygenase (COX)-2, and TNF was tested in the metaphyseal region (Smith et al., 2006) (Figure 1). Chongwatpol P. et al. illuminated that LPS substantially decreased trabecular bone volume, lumbar vertebra bone mineral density, and the number of the vertebral body compared with both zinc inadequate and adequate without LPS groups (Chongwatpol et al., 2015).

2.2 | Bile acid

Primary bile acids were conjugated with taurine or glycine to make up bile salts in liver, then they were secreted into the small intestine. Ninety-five percent of bile salts are transported back to the liver and enter "gut-liver axis" when reaching the ileum. Roughly 400~600 mg of bile salts reach the large intestine (Figure 2). They experience a variety of anaerobic bacterial biological transformation to secondary bile acids, primarily lithodeoxycholic acid and deoxycholic acid (Donerner, Takamine, Lavoie, Mallonee, & Hylemon, 1997; Wells & Hylemon, 2000). The gut microbiome plays a significant role in the bile acid metabolism (Ridlon, Kang, Hylemon, & Bajaj, 2014). Through farnesoid X receptor (FXR) and G protein-coupled bile acid

**FIGURE 2** (a) Bile acids experienced a "gut-liver axis" and were transformed to the secondary bile acid under the influence of anaerobic bacteria. (b) Through FXP and TGR5 signaling, intestinal microbial components can change the amount and the type of secondary bile acid. (c) Some types of secondary bile acid are agonists of the membrane-bound G-protein-coupled receptor (TGR5). Stimulation of TGR5 can increase the production of glucagon-like peptide-1 (GLP-1), a kind of enterogenous hormone, which can active thyroid C cells proliferation and promote the secretion of calcitonin, thus inhibiting bone resorption. GLP-1 also can stimulate the proliferation of osteoblast and inhibit osteoclast. (Sandoval & D’Alessio, 2015) (d) LCA can damage osteoblasts mitochondrial and reduce cell viability. As a mild VDR ligand, LCA can reduce the gene expression of osteocalcin and RANKL.
receptor 5 (TGR5) signaling, intestinal microbial components can change the amount and the type of secondary bile acid, thus producing different metabolic effects (Figure 2). The enzymes contained in the deconjugation, epimerization, and dehydroxylation of bile acids are existed in many species (Labbe, Ganopolsky, Martoni, Prakash, & Jones, 2014). The variety of food can affect intestinal microbiota and the metabolism of bile acids. In mice consumed mung bean protein (MPI), the enhancement of cecum and fecal cistern was observed. These effects were eliminated in GF mice, suggesting that their effects depended on the presence of microbiota. Analysis of the 16s-rRNA gene sequence had revealed that intake of MPI can also cause drastic changes in intestinal microbiota, such as the reduction of firmicutes and the expansion of phylum bacteroidetes (Nakatani et al., 2018). In addition, oral administration of vancomycin can reduce secondary bile acid and worse insulin sensitivity by changing intestinal microbiota (Vrieze et al., 2014). It is worth mentioning that monohydroxylated secondary lithocholic acid (LCA) can be as a kind of vitamin D receptor (VDR) ligand synthesized primarily by 7-dehydroxylation of intestinal bacteria (Adachi et al., 2005).

It is reported that 1,25-dihydroxyvitamin D3 played an important role in bone integrity (van Leeuwen, Van, Gj, & Pols, 2001) and mineral balance (Sutton & Macdonald, 2003). The biological effects of vitamin D are regulated by its receptor, the VDR, which is one of the steroid receptors that can control the biological effects of multiple hormones. Vitamin D also regulates genes coding for osteoprotein, (Margolis & Christakos, 2010; Shen & Christakos, 2005) osteocalcin (Ozono, Liao, Kerner, Scott, & Pike, 1990), and the receptor activator of NF-kb ligand (RANKL), (Kim, Yamazaki, Zella, Sheyde, & Pike, 2006; Kitazawa, Mori, Yamaguchi, Kondo, & Kitazawa, 2008) which directly controls the bone turnover. Due to the toxicity of LCA in osteoblasts and the ability of binding to VDR, it seems that LCA may affect bone metabolism (Figure 2). Excessive LCA may play a role in the pathogenesis of osteoporosis. In the hamster model, LCA caused osteoblasts mitochondrial damage, which reduced cell viability, (Ceryk, Bouscarel, Malavolti, & Fromm, 1998) although the concentration is as low as 100IM (Ruiz-Gaspa et al., 2010). In many tissues, vitamin D activates the enzyme 1a, 25-dihydroxyvitamin D3 24-hydroxylase (CYP24A1) through a vitamin D-dependent pathway, regulating its own metabolism. In the condition of no effects on cell viability, LCA significantly reduced CYP24A1 expression by 72% (Kim et al., 2006). CYP24A1 contains two vitamin D reaction elements (VDREs) area. These sequences depend on vitamin D to induce the gene expression (Tashiro, Abe, Oue, Yasui, & Ryoji, 2004; Tashiro, Ishii, & Ryoji, 2007). The effect of LCA on CYP24A1 is produced by the VDREs that were located in CYP24A1 promoter region, which further indicates that LCA is a mild analog of vitamin D. In addition to the ability of LCA catabolism of vitamin D, LCA reduces the other two genes expression, such as osteocalcin, closely associated with bone formation, and RANKL which is expressed in osteoblasts and regulated osteoclast formation. LCA reduced the ability of vitamin D to activate osteocalcin and RANKL genes by 79% and 56%, respectively (Ruiz-Gaspa et al., 2010).

2.3 Short-chain fatty acids (SCFAs)

Bacteria in the colon can ferment indigestible carbohydrates into SCFAs, including acetate, propionate, and butyrate. In addition, the fermentation of amino acids by intestinal bacteria also produces SCFAs. Protein fermentation accounts for 17%-38% of SCFAs in cecum and sigmoid colon (Mccabe, Britton, & Parmeswaran, 2015). For enterocytes, butyrate is a significant energy source, while propionate and acetate are mainly absorbed by liver and used as a material source for gluconeogenesis (Den et al., 2013). Acting as signaling molecules, SCFAs can activate AMP kinase and free fatty acid receptors 2 and 3 (FFAR2 and 3), termed as G-protein-coupled receptors 43 and 41 (Zhang et al., 2015). SCFAs can inhibit lipogenesis and stimulate fatty acid oxidation (Den et al., 2013), which can prevent from the development of nonalcoholic fatty liver disease.

The oligosaccharide diet increases the production of SCFAs and changes the microbial composition, indicating the close relationship between microbiota, dietary fiber content, and SCFAs (Smiricky-Tjardes, Grieshop, Flickinger, Bauer, & Jr, 2003). SCFAs products varied according to the types of amino acid matrix and intestinal bacteria (Dai, Jing, Wu, & Zhu, 2010; Smith & Macfarlane, 1997), such as Clostridium, Bifidobacterium, and Lactobacillus, which mainly produce SCFAs (Den et al., 2013). Bone mineral density increased significantly and briefly after low-dose antibiotic treatment in conventionally fed mice, suggesting that bacterial microbiota may play a role in bone catabolism (Cho et al., 2012). Consistent with the study, Sjogren and his colleagues found that colonizing young adult GF animals with bacteria can lead to bone loss of trabecular and the proliferation of osteoclast (Sjogren et al., 2012). However, some other studies suggested that administration of beneficial microorganisms can moderately increase bone density (Britton et al., 2014; Mccabe et al., 2015; Mccabe, Irwin, Schaefer, & Britton, 2013; Parvaneh, Jamaluddin, Karimi, & Erfani, 2014; Weaver, 2015; Zhang et al., 2015) and prevent from bone loss during menopause (Li et al., 2016). The above inconsistent results may reflect the differences in the composition of microbiota, animal species, and gender in these studies. Bone turnover is composed of bone resorption and bone formation. When bone formation induced by bacteria is not enough to offset bone resorption, it will lead to bone catabolism. Systematic insulin-like growth factor 1 (IGF-1), a hormone known to have an effect on bone growth, significantly increased after microbiota transplantation. Exogenous IGF-1 promoted the growth of longitudinal femoral (Yakar et al., 2002). Insulin-like growth factor 1 receptor deletion model indicated that IGF-1 plays a vital role in the mature growth plate and in the process of the formation of secondary ossification center (Wang et al., 2011). Compared with GF mice, circulating IGF-1 increased in both short-term and long-term microbiota transplantation mice. It seems to be contrary to the result of Sjogren and his colleagues. In Sjogren's study, they colonized 3-week-old female GF C57Bl6/J mice with normal gut microbiota. Four weeks after colonization, the trabecular BMD had been significantly reduced compared with GF counterparts. However, in Jing's study, they
colonized 2-month-old female GF CB6F1 mice with normal gut microbiota. Four weeks after colonization, trabecular bone mass was decreased compared with GF controls, which was the same as Sjogren's study. Furthermore, Jing also found that the serum C-terminal telopeptides of type I collagen (CTX-I), N-terminal pro-peptide (P1NP), and IGF-1 were increased in colonized mice. This suggests that the bone resorption promoting effects of colonization overtake the effect on bone formation. However, 8 months after colonization, the effect on bone formation was dominant, leading to increased bone mineral density of longitudinal and radial bones (Yan et al., 2016). The duration of colonization, age, and strain of the mice may be the explanation of the contrary results in these two studies. In short-term transplantation, IGF-1 stimulated bone resorption dominantly, which will cause bone loss. However, IGF-1 stimulated bone formation dominantly over a longer period, resulting in an increase of bone mass (Schwarzer et al., 2017). Antibiotic treatment reduced the level of SCFAs, IGF-1, and P1NP. After given SCFAs, the levels of systematic IGF-1 and bone mass in the antibiotics-treated mice were the same as the nonantibiotic mice. Therefore, the production of SCFAs may be a mechanism by which microbial community increased the serum level of IGF-1. Compared with mice treated with antibiotics, SCFAs supplementation increased the production of fat pad IGF-1. Serum insulin-like growth factor binding protein 3 (IGFBP3) was not changed. In addition, reduction of trabecular bone mass was observed after 4 months of SCFAs supplementation, similar to short-term microbiota transplantation (Yan et al., 2016). Other mechanisms of SCFAs are shown in Figure 3.

### EFFECT OF MICROBIOTA ON IMMUNE SYSTEM AND BONE HOMEOSTASIS

Recognizing that the microbiota and immune system are significant to the balance in the skeleton means that evolvement from the field of bone immunology to “osteomicrobiology,” a term coined by Ohlsson et al (Ohlsson & Sjögren, 2015). Intestinal microbiota is essential for the function and maturation of immune system. The relation between the microbiota and the skeleton was first discovered in 2012 (Sjogren et al., 2012). Compared with the control group under normal conditions, the amount of trabecular bone was increased in mice under sterile conditions. Since this phenomenon was overturned by colonization of the gut microbiota from conventionally fed mice, the evidence was compelling that results are not on account of the innate abnormalities of GF mice. This study also found that the number of CD4 T cells and TNF in bone marrow was lower in GF mice. In another study, probiotics seemed to increase bone density and reduce intestinal inflammation in men rather than women (Mccabe et al., 2013). The close relationship between bone loss and inflammatory conditions has long been valued by clinicians (Mbalaviele, Novack, Schett, & Teitelbaum, 2017; Redlich & Smolen, 2012). Specific strains had effects on specific immune cells, (Goto et al.,

![Figure 3](image-url)
2014; Ivanov et al., 2009) and new insights have been developed on the effect of the whole microbiome on the immune system.

3.1 | Th17 and Treg cells

T cells are a heterogeneous group of cells. By function, it can be classified into helper T cells (Th cells), inhibitory T cells (Ts cells), cytotoxic T cells (CTL or Tc cells), and delayed hypersensitivity T cells (TDTH cells).

Producing interleukin-17 (IL-17) and many other effector cytokines, such as IL-22, Th17 cells are important for activating innate immune mechanism, including inducing epithelial cells to produce antimicrobial peptides and recruiting neutrophils. Th17 cells play a significant role in mucosal resist against bacteria and fungi (Korn, Bettelli, Oukka, & Kuchroo, 2009). Transplantation of segmented filamentous bacteria (SFB) into GF mice increased the number of Th17 cells and mildly increased Th1 cells (Goto et al., 2014; Ivanov et al., 2009) SFB seems to penetrate the mucous layer of the terminal ileum, contact with epithelial cells, and induce actin aggregation, possibly leading to Th17 polarization signals in the lamina propria. Little is known that Th17 polarizing signaling pathways can be initiated by SFB. It is likely that SFB affects the expression of antimicrobials proteins RegIIIg and molecules in epithelial cell, which are involved in Th17 cell polarization. In addition, studies have shown that MHCII-dependent antigen presentation of SFB antigens by intestinal dendritic cells (DCs) is crucial for Th17 cell induction (Goto et al., 2014) (Figure 4).

CD4+FOXP3+ Treg cells are stable in the intestinal mucosa and have an effect on the intestinal and systemic immune system. In GF mice, Treg cells and IL-10 were significantly reduced (Atarashi et al., 2011; Geuking et al., 2011). In a study, researchers separated 17 strains of bacteria, which can enhance the expansion of Treg cells and induce significant anti-inflammatory molecules, such as IL-10 and inducible T-cell stimulator (ICOS). These 17 strains provided

**FIGURE 4**  (a) Th17 cells produce a pro-osteoclastogenic effect. SFB influenced the expression of antimicrobial proteins RegIIIg participating in Th17 cell polarization; SFB induced Th17 cell differentiation by intestinal epithelial cells production of serum amyloid A that might affect DC cytokine; Th17 cell differentiation depended on MHCII-dependent antigen presentation of SFB antigens by intestinal dendritic cells; Bacteroides fragilis prevented the differentiation of Th17 cells by polysaccharide A. (b) Tregs regulated the formation of osteoclast. Treg cell differentiation was induced by short-chain fatty acids, leading to epigenetic changes to stabilize the program; Clostridium genus provided bacterial antigens and a TGF-β-rich environment, resulting in the expansion of systemic Tregs. (c) NLRs bind to bacterial peptides and attract receptor interaction protein (RIP2), stimulating the NF-kB signaling pathway, which can induce osteoclastogenesis through chemokines and cytokines.
a TGF-β rich environment and bacterial antigen, thus inducing the expansion and differentiation of Treg cells (Figure 4). Genomic sequencing showed that the 17 strains belonged to IV, XIVa, and XVIII of Clostridia. The Clostridias were lack of virulence factors and significant toxins (Oshima, 2013).

Transplantation of the GF mice with IV and XIVa of Clostridia, separated from mouse droppings, resulted in an increase in systemic and the lamina propria Treg cells. Compared with thymus Tregs, the peripheral Tregs have a phenotypic characteristic, namely they react to TGF-β and retinoic acid (Atarashi et al., 2011). Furthermore, the induction effect of intestinal microbiota on systemic Th17 and Treg cells was also confirmed (Atarashi et al., 2011; Berer et al., 2011; Yun & Gordon, 2011). Immune function was regulated by bacterial metabolites, mainly short-chain fatty acids (SCFAs). It has been reported that butyrate exerts immunomodulatory effect on intestinal macrophages, induces Treg cell differentiation (Furusawa et al., 2013) (Figure 3).

Th17 cells are essential for estrogen-deficient bone loss. Th17 cells are a subset of CD4⁺T cells, which can produce a pro-osteoclastogenesis effect. In women, the increase of serum IL-17 is closely related to osteoporosis (Molnar, Bohaty, & Somogyine-Vári, 2014; Molnár, Bohaty, & Somogyiny-Vári, 2014; Zhang et al., 2014). The elimination of IL17 (Deselm et al., 2012) or the use of anti-IL17 antibody (Tyagi et al., 2014) may prevent from bone loss (Figure 4). Treg cells deficiency and inactivation are associated with some chronic inflammatory diseases. Tregs regulate the formation of osteoclast by secretion of IL-4, IL-10, and TGF-β (Kelchtermans et al., 2009; Kim et al., 2007; Luo, Wang, Sun, & Li, 2011; Zaiss et al., 2007) (Figure 4) and blocking bone resorption (Yuan et al., 2010). Importantly, it is well known that estrogen directly increases the relative number of Tregs, (Tai et al., 2008) which prevents from ovx-induced bone loss (Zaiss et al., 2010).

### 3.2 NOD1 and NOD2 signaling

The innate immune system can recognize a variety of pathogen, which is the body’s first defense against extraneous pathogenic microorganisms. It recognizes pathogens through specific pattern recognition receptors (PRRs), including the nod-like receptor family (NLR) in the cytoplasm. On the cell surface, innate immune system in the intestines identified bacteria by PRRs, such as toll-like receptor (TLR) family and other signaling pathways. Most of the TLR signals are adjusted through MYD88 protein to stimulate the mitogen-activated protein (MAP) kinase and proinflammatory signals of NF-κB (Kufer & Sansonetti, 2007). In the cytoplasm, bacterial detection is carried out by NLRs, NOD1, and NOD2. They bind to bacterial peptides and attract a common protein kinase, receptor-interaction protein (RIP2) which stimulates the NF-κB signaling pathway, leading to gene expression of chemokines and cytokines.

NOD1 was found in many types of cells. The proinflammatory signal was induced by recognition of peptidoglycan mainly found in gram-negative bacteria (Clarke et al., 2010). NOD2 was widely expressed in nonhematopoietic cells, bone marrow derived cells, and lymphocytes (Nigro, Rossi, Commere, Jay, & Sansonetti, 2014; Ogura et al., 2001). NOD2 can bind to all types of peptidoglycan that is found in gram-positive and gram-negative bacteria, inducing inflammatory response by activation of NLRs (Figure 4). Compared with conventionally fed Myd88⁻/⁻ mice, the cortical bone mass of GF Myd88⁻/⁻ mice increased significantly, indicating that the influence of GF mice on bone was independent of Myd88 protein. In contrast, the cortical bone mass of GF mice that specifically deactivated NOD1 or NOD2 did not show a significant increase, indicating that the influence of GF mice on cortical bone mineral density depended on the signals of NOD1 or NOD2 (Ohlsson et al., 2017). The effect of NOD2 on bone resorption was verified in microbiota-induced periodontitis model. In NOD2-deficient mice, bone resorption and the number of osteoclast were significantly reduced (Prates et al., 2014; Souza et al., 2016). Additionally, bone marrow macrophage that extracted from NOD2-defective mice would form less osteoclast cells than that from wild-type mice, which suggested that bone resorption induced by bacteria was relied on NOD2 signal. Furthermore, NOD2 ligand could induce osteoclastogenesis when the RANKL gene expression increased in osteoblasts. (Yang et al., 2005)

### 3.3 Wnt signaling

Wnt signaling pathway widely exists in invertebrates and vertebrates. It is a highly conservative signaling pathway in the evolution of species. Wnt signaling plays a vital role in the early development of animal embryos, organ formation, tissue regeneration, and other physiological processes. Wnt/β-catenin signaling can be activated by some bacteria such as the *Fusobacterium nucleatum* and *Bacteroides fragilis* (Rubinstein et al., 2013; Wu, Morin, Maouyo, & Sears, 2003). Intestinal microbiota can polarize colon macrophages into M1 state, thus producing endogenous inflammatory cytokines (Yang, Wang, Moore, Lightfoot, & Huycke, 2012; Yang et al., 2013). These events were called microbial-induced bystander effects (MIBE). TNF contributes to MIBE and activated Wnt/β-catenin signaling (Oguma et al., 2008). One study has shown that TNF activated Wnt/β-catenin by inducing Wnt3 and inhibiting Wnt inhibitory factor 1 (WIF1). In normal colonic epithelial cells, the basal Wnt level is restricted by WIF1, which was phosphorylated and degraded continuously by protease ubiquitination (Wang, Yang, & Huycke, 2017). β-Catenin is a key component of the Wnt/β-catenin signaling pathway, which regulates Wnt target gene transcription when activated by various Wnt ligands (wnt10b, wnt1, wnt3, etc.). Osteoblast function was regulated by Wnt/β-catenin signaling in almost all aspects from infancy to maturity (Hill, Später, Takeito, Birchmeier, & Hartmann, 2005; Song et al., 2012). Therefore, β-catenin is the key target to explore the function of this pathway on osteoblast. A large number of mouse models have shown that from immature (Day, Guo, Garrett-Beal, & Yang, 2005) to mature stage, (Kramer et al., 2010) the depletion of β-catenin can inhibit the differentiation of osteoblasts and increase the differentiation of osteoclasts, resulting in decreasing bone mass. Wnt binds to the frizzled protein (Fz) receptor on the surface of osteoblasts, resulting in stabilizing of intracellular β-catenin. When associated
with the T-cell factor/lymphoid enhancer factor (LEF/TCF) transcription factors, β-catenin activates the transcription of osteoprotegerin (OPG) in osteoblasts, thereby reducing bone absorption (Nd et al., 2005). As previously shown, the loss of β-catenin leads to a significant reduction in bone mass as a result of decreased bone formation and increased bone resorption (Holmen et al., 2005).

4 | EFFECT OF MICROBIOTA ON HORMONE AND BONE HOMEOSTASIS

Since Lyt and his colleagues observed that stress-induced neuroendocrine hormones affect the growth of bacteria, the relationship between microbes and endocrine has been concerned (Cooper, Knowles, Brown, Mcqguril, & Dooley, 1992).

So far, it has been found that intestinal microbiota is closely related to a large number of hormone secretion, such as gut microbiota produced serotonin, (Freestone & Lyte, 2008) Lactobacillus produced gamma-aminobutyric acid (GABA), low level of corticosterone in probiotics-treated mice, (Asano et al., 2012) decreased levels of insulin, (Karllson et al., 2013) low glucagon-like peptide-1 (GLP-1) levels in antibiotic-treated mice, (Wichmann et al., 2013), and probiotics (Storelli et al., 2011). As is known to all, GLP-1 plays an important role in bone turnover. Similarly, intestinal microbiota also plays a significant role in bone turnover through many other hormones.

4.1 | Sex hormones

There are many conflicting results in studies on the association between intestinal microbiota and sex hormones in healthy people (Arunugam et al., 2014; Ding & Schloss, 2014; Schnorr et al., 2014). In males, Ruminococcus, Bacteroides, Eubacterium, and Blautia were in a greater abundance, while Treponema in females. However, these differences may be on account of the specific way of life and cultural factors related to gender, rather than sex hormones. It is reported that intestinal microbiota may affect the balance of steroids. Certain species have the ability to metabolize sex hormones and affect their activity (Lombardi, Goldin, Boutin, & Gorbach, 1978). For example, the intestinal symbiotic Clostridium scindens converted glucocorticoids into androgens with hydroxysteroid hydrodase and other enzymes (Ridlon et al., 2013). Slacksia sp. is a common member of the intestinal microbiota, which can influence the production of estrogen. The effect of intestinal microbiota on sex hormones conversion has been established.

Furthermore, numerous studies have confirmed the role of estrogen in bone metabolism. The estrogen receptors (ERs) are expressed in osteocytes, (Tomkinson, Gevers, Wit, Reeve, & Noble, 1998) osteoblasts, (Komm et al., 1988) osteoclasts (Oursler, Osdoby, Pyfferen, Riggs, & Spelsberg, 1991), and bone marrow stromal cells. Estrogen was able to induce the apoptosis of osteoclasts and inhibit the apoptosis of osteoblasts (Kousteni et al., 2001; Sims et al., 2003). Bone turnover cycle was activated more frequently in an estrogen deficiency environment (Erik, Langdahl, Vesterby, Rungby, & Kassem, 1999). In addition to directly affect bone cells, estrogen regulated oxidative stress and the immune system to influence bone turnover cycle. Estrogen deletion reduced the capacity of mature osteoblasts by stimulating output of proinflammatory cytokines such as TNF, IL-7, and IL-1 (Gilbert et al., 2000; Weitzmann, Roggia, Toraldo, Weitzmann, & Pacifici, 2002). T cells are important source of TNF in ovariectomized mice and postmenopausal women (Adeel et al., 2013). In the case of T cell-deficient mice, ovariectomies do not induce bone loss (Gao et al., 2007). TNF plays an important role in bone loss in ovariectomized mice. The key mechanisms of TNF in stimulating bone resorption were the activation of receptor activator of NF-kB (RANK) (Cenci et al., 2000) and the induction of Th17 cells (Sugita et al., 2012). Treatment with IL-1 and TNF inhibitors prevented from the increase of bone resorption due to estrogen deficiency (Charatcharoenwitthaya, Kholsa, Atkinson, Mccready, & Riggs, 2007). Studies have shown that sex hormone-mediated bone metabolism not only reacted through ERs, but also through androgen receptors (ARs) (Kousteni et al., 2001). Androgen inhibits bone resorption via ARs in osteoblasts and cancellous compartment. It is essential for trabecular bone accrual during growth. Furthermore, ARs signaling takes a significant role on protecting cortical thickness and strength in aging (Jardi et al., 2018). The mechanisms by which sex hormones affect bone metabolism are greatly complex. More human studies are needed to confirm whether intestinal microbiota can influence bone metabolism directly by regulating the systematic sex hormones transformation.

4.2 | Serotonin (5-hydroxytryptamine, 5-HT)

Serotonin, produced in the circulation, inhibits bone formation. By contrast, produced in the brain like a neurotransmitter, serotonin has a positive effect on bone mass by increasing bone formation and inhibiting bone absorption (Ducy & Karsenty, 2010) (Figure 5). 5-HT is produced by specialized cells called enterochromaffin cells (ECs), mucosal mast cells, and intestinal muscle nerves (Gershon & Tack, 2007). More than 90% of 5-HT are synthesized in the human gut. There are 14 different receptor subtypes in intestinal epithelial cells, (Hoffman et al., 2012) immune cells (Baganz & Blakely, 2013), and intestinal neurons. (Mawe & Hoffman, 2013) Intestinal-derived 5-HT regulated different functions, including intestinal movement, secretion reactions, immune response, (Baganz & Blakely, 2013) platelet aggregation, (Mercado et al., 2013) cardiac function, and bone development (Chabbiachengli et al., 2012). There are two different tryptophan hydroxylase isoenzymes Tph1 and Tph2, which regulate the synthesis of neurogenic and non-neurogenic 5-HT. Recent studies had focused on the role of intestinal microbiota in regulating the blood levels of 5-HT. Studies have proved that corynebacterium, streptococcus, and E.coli may produce 5-HT in culture (Roshchina, 2016). The serum concentration of 5-HT in GF mice was significantly lower than that in normal mice (Sjogren et al., 2012). Interestingly, some bacteria in healthy mice and humans can regulate the serum level of 5-HT (Tsavkelova,
Spore-forming microbes (Sp) can fully regulate the levels of 5-HT in serum, colon, and fecal (Figure 5). It is found that the expression level of Tph1 (the rate-limiting enzyme for 5-HT biosynthesis) was greatly reduced in the colon of GF mice, but the 5-HT packaging, release, and catabolic enzymes had no difference (Yano et al., 2015). The 5-HT transporter SLC6A4 was also highly expressed in the colon of GF mice, and was widely synthesized by intestinal cells to achieve 5-HT absorption. This may reflect the host's compensatory response to lack of 5-HT (Wade et al., 1996). In GF mice, transplantation of intestinal microbiota can restore the levels of 5-HT in serum and colon at every age. At early stage, the effect of intestinal microbiota transplantation was more obvious (Yano et al., 2015). The level of 5-HT was consistent with that of GF mice after separate transplantation of SFB or fragile bacteroidetes. However, transplantation of Sp microbes into GF mice completely restored serum and colon 5-HT levels (Stefka et al., 2014). It is interesting that the level of 5-HT in GF mice was also restored after transplanted with Sp microbes from healthy human, suggesting that the adjustment role of Sp microbes in 5-HT level can transfer between human and mice (Yano et al., 2015).

There are three serotonin receptors expressed in osteoblasts, Htr1b, Htr2a, and Htr2b. Inhibition of Htr2b activity can reduce bone formation leading to a decreased bone density in female mice (Collet et al., 2008). The combination of serotonin with Htr1b on the surface of osteoblasts inhibited the production of cAMP and the phosphorylation of PKA-mediated cAMP reaction element (CREB), which result in a decreased expression of cyclin genes and a reduced osteoblast proliferation. Similarly, osteoblast specific inhibition of CREB can lead to low bone formation phenotype and low bone mass. In Htr1b−/− mice, the downregulated level of CREB normalize their high bone mass phenotype. In addition, in vitro and vivo gene expression analysis confirmed that cell cycle proteins D1, D2, and E1 were the transcription genes for CREB under the regulation of gut-derived serotonin (Figure 5). Thus, these phenomena suggested that the direct targets of gut-derived serotonin are osteoblasts, while Htr1b/PKA/CREB/cyclins signaling regulates its proliferation (Yadav et al., 2008).
4.3 | Leptin

Leptin is a kind of hormone derived from adipocyte specific to vertebrate. It can regulate physiological processes such as bone mass, energy expenditure, and appetite. The long branch of vagus nerve regulates the interaction between brain and gut microbiota, known as the "gut-brain axis". Some evidence links microbiota to the level of leptin. First of all, use of vancomycin can cause a sharp decrease in leptin levels in rats (Lam et al., 2012). Secondly, a large number of bacteria species (such as Lactococcus, Mucispirillum, Lactobacillus, and Bifidobacterium) are positively correlated with peripheral leptin concentrations, while other bacteria species (such as Clostridium, Prevotella, Bacteroides, and Allobaculum) are negatively correlated with leptin levels (Queipoortuño et al., 2013) (Figure 5). Other evidence suggested that L.plantarum inhibited leptin by reducing the size of fat cells in white adipose tissue (Takemura, Okubo, & Sonoyma, 2010). The use of probiotics in a group of smokers reduced their leptin levels (Naruszewicz, Johansson, Zapolskadownar, & Bukowska, 2002).

Leptin receptor (ObRb) is expressed in the same brainstem neuron of the serotonin producing raphe nucleus (Scott et al., 2009). The combination of leptin and ObRb inhibited the expression of Tph2 gene and decreased the serotonin release of synthetic brain stem neurons (Charnay et al., 2000). Compared with mice deleting ObRb in the arcuate (ARC) and ventral hypothalamus (VMH), mice deleting ObRb serotoninergic neurons showed high bone mass phenotypes (Yadav et al., 2009). Serotonin receptors Htr2c are expressed on VMH nucleus. The loss of Htr2c receptors in these neurons results in severe bone loss. This phenomenon was caused by the downregulation of bone formation and upregulation of bone resorption related to an increased sympathetic nerve activity (Yadav et al., 2009) (Figure 5). Brain serotonin plays an important role in VMH neurons through Htr2c to reduce sympathetic activity and contribute to the beneficial effect on bone mass. Leptin-dependent central regulation of bone mass depends on the regulation of sympathetic nervous system through brain serotonin (Karsenty, 2006).

In conclusion, intestinal microbiota components can regulate bone metabolism via influencing the host metabolism, immunity, and endocrine environment, which may provide new ideas and targets for the clinical treatment of osteoporosis. However, most of findings need to be further validated in human studies as they are mainly drawn from animal studies.

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CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

AUTHORS CONTRIBUTION

Lishan Li (First Author) and Shitao Rao (Co-first author) conceptualized the manuscript and wrote the first draft. Yanzhen Cheng, Xiaoqun Zhuo, Caihong Deng, Ningning Xu, Hua Zhang and Li Yang contributed to the conception of the manuscript. Hua Zhang and Li Yang thoroughly reviewed the manuscript. All authors approved the final version of the manuscript.

ETHICS STATEMENT

None required.

DATA ACCESSIBILITY

None required. All figures used in this review article are original.

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