



# Linking the relation between gut microbiota and glucocorticoid-induced osteoporosis

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## Abstract

Osteoporosis (OP) is the most prevalent metabolic bone disease, characterized by the low bone mass and microarchitectural deterioration of bone tissue. Glucocorticoid (GC) clinically acts as one of the anti-inflammatory, immune-modulating, and therapeutic drugs, whereas the long-term use of GC may cause rapid bone resorption, followed by prolonged and profound suppression of bone formation, resulting in the GC-induced OP (GIOP). GIOP ranks the first among secondary OP and is a pivotal risk for fracture, as well as high disability rate and mortality, at both societal and personal levels, vital costs. Gut microbiota (GM), known as the “second gene pool” of human body, is highly correlated with maintaining the bone mass and bone quality, and the relation between GM and bone metabolism has gradually become a research hotspot. Herein, combined with recent studies and based on the cross-linking relationship between GM and OP, this review is aimed to discuss the potential mechanisms of GM and its metabolites on the OP, as well as the moderating effects of GC on GM, thereby providing an emerging thought for prevention and treatment of GIOP.

**Keywords** Gut microbiota · Gut · Bone · Glucocorticoid-induced osteoporosis

## Introduction

Osteoporosis (OP) is the most prevalent metabolic bone disease, characterized by low bone mass and microarchitectural deterioration of bone tissue [1, 2]. According to data provided

by International Osteoporosis Foundation (IOF), more than 200 million people worldwide suffer from OP and there is an average fracture caused by OP every 3 s, and the incidence of hip fracture has been increasing in the world since 1990, which is expected to increase by 240% in women and 310% in men by 2050, and about 50% of osteoporotic fractures might occur again [3–6]. Glucocorticoid (GC) clinically acts as the anti-inflammatory, immune-modulating therapeutic drugs used to manage inflammatory diseases including inflammatory bowel disease (IBD), allergic conditions, bronchial asthma, rheumatoid arthritis (RA), ankylosing spondylitis, and chronic renal diseases, cancers, as well as severe corona virus disease 2019 (COVID-19) cases [7]. However, long-term application of GC may cause rapid bone resorption, followed by prolonged and profound suppression of bone formation, leading to an “imminent risk of fracture” [8–11]. More than 41.4% of patients developed the OP during the long-term treatment of GC [12]. A recent claim-based assessment on the costs of adverse events related to GC, established an incremental cost of \$3201 per fracture at the cumulative doses greater than 1800 mg. GC is the cause of disability, mortality and, at both societal and personal level, significant costs [13, 14]. Though antiresorptive and anabolic drugs could help to reduce the OP due to GC, GC-induced OP (GIOP) ranks the first among secondary OP

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and is a significant risk for fractures [9, 10]. Thus, the in-depth studies of cross-linking relationship and development of corresponding targeted therapy strategies have become the key to the prevention and treatment of GIOP.

In general, GIOP is most common in drug-induced secondary OP [15]. The etiology of GIOP is mainly related to the increase of osteoblast apoptosis and osteoclast activity. Physiologically, cortisol secreted by the adrenal cortex is an essential hormone for the differentiation and functional regulation of osteoblastic lineage cells and osteoclastic lineage cells [16, 17]. However, excessive physiological dose of GC and its analogues might have apparent adverse influences on the development, growth, and metabolism of bone tissues [18]. Histologically, the osteoblast apoptosis and inhibition of osteoblast function could be observed, while the number of bone remodeling units increases, bone resorption lacunae increase, the bone formation is insufficient, and the bone trabecular thickness decreases, perforates, or disappears [19]. Additionally, different from primary OP, GIOP is mainly characterized by following three characteristics: (1) The influences of GC on the bone tissues are mainly characterized by the obvious activation of receptor activator for nuclear factor- $\kappa$ B ligand (RANKL) and enhanced bone resorption, but no corresponding increase in bone formation occurred during this period; (2) After a period of adaptation (approximately several weeks), the bone formation remains at a low level, while the bone resorption decreases; (3) The risk of fracture enhances rapidly, and in a dose-dependent manner after the application of GC [20–22].

In recent years, the relationship between GM and human diseases has been focused on. Gut microbiota (GM) is the community of microbes (bacteria, fungi, viruses, etc.) residing in the host gastrointestinal tract, also known as the “second gene pool” of the human body, and the number of genes in its genome is about 150 times as much as the total number of genes in human genome, approximately 10 trillion [23, 24]. Increasing researches have indicated a complex role of the gastrointestinal tract in keeping bone health through a “gut-bone” axis, in which the several mechanisms have been proposed [25–27]. Animal experiments have shown that GM can regulate bone mass by altering immune status, intestinal calcium absorption, and affecting osteoclasts-mediated bone resorption. Taken together, GM is highly correlated with maintaining bone mass and bone quality [28–30]. It would seem imperative to understand the mechanisms by which GM affects the development of GIOP to identify drug targets and design better therapies for this disease.

## The relationship between GM and OP

GM are known as the “second gene pool” of the human body, encoding 150-fold more genes than human genome, and about 10 trillion bacteria colonizing in the human

gastrointestinal tract interacts with the host [23, 28]. GM is referred to as a commensal, symbiotic, and pathogenic microorganism living in our intestines, playing an essential role in endocrine system, enteric nervous system, immune system, nutrient absorption and production, metabolic balance, gut–brain–bone axis, and resistance to pathogens at different period of ages [31–33]. Nowadays, the knowledge of the complex interaction between GM and health outcomes is a novel and rapidly expanding field [31]. In recent studies, it has been observed that GM alterations associated with reduced bone mineral density (BMD) in older adults [34, 35]. There is an increasing number of human and animal studies suggesting that GM can exert effects in skeletal system as it modulates gut permeability, hormonal secretion, and immune response, and stimulates calcium and vitamin D absorption [36].

Moreover, GM is a critical regulator of bone remodeling mediated by the osteoclasts with bone resorption function and the osteoblasts with bone formation function. Therein, bone strength refers to the ability of bones to respond to mechanical load, and GM may play a key role in the formation of differences in bone strength [37]. Long-term changes in GM during growth and development may not only result in the changes in bone mass, but also impair the biomechanical properties of bone [38]. Several studies have reported the alterations in the properties of bone collagen associated with bone fragility, such as changes in biochemical properties and protein structure [39, 40]. The supplementation of specific probiotics in OP-related mice models can improve BMD and enhance bone heterogeneity [41]. This is of great practical value, as most of the current anti-OP drug candidates (such as the bisphosphonate, calcitonin, cathepsin K inhibitors, and estrogen) may reduce the bone heterogeneity, and thereby enhancing the risk of bone fragility and fractures [42, 43]. Besides, Chen et al. [44] also observed that feeding GC-treated mice in the same nest with healthy mice, or transplanting the GM of healthy mice to the GC-treated mice could significantly reduce the decrease of bone mass, bone microstructure destruction, decreased angiogenesis, decrease in the number of osteoblasts and increase in apoptotic cells induced by GC.

From the aspect of clinical researches, Yang et al. [45] conducted the 16 S ribosomal RNA (16 S rRNA) sequencing analysis of the feces from 132 postmenopausal women, including patients with OP, patients with osteopenia and the individuals in the control group, and observed that the composition and diversity of GM in OP group and control group were significantly different, reflecting that the abundance of *Fusicatenibacter*, *Lachnoclostridium*, and *Megamonas spp.* was higher in the OP group. In a randomized, double-blind, placebo-controlled, multi-center trial, Jansson et al. [46] applied a mix of 3 kinds of *Lactobacillus* strains (*Lactobacillus paracasei* DSM 13434, *Lactobacillus plantarum* DSM

15312, and *Lactobacillus plantarum* DSM 15313) at a dose of  $1 \times 10^{10}$  CFU per day for the postmenopausal women, and it turned out that the supplementation of *Lactobacillus* strains reduced the bone loss in postmenopausal women compared with placebo, indicating the benefits of probiotics and prebiotics for bone metabolism. Morato-Martínez et al. [47] suggested that regular consumption of a dairy product to reconstitute enriched with bioactive nutrients significantly enhanced dietary intake of calcium and vitamin D, thus improving bone health-related markers in menopausal women at risk of OP without pharmacological treatment.

As for the animals' studies, by constructing the rat models of postmenopausal OP induced by the ovariectomy (OVX), Li et al. [23] suggested that the metabolites of GM were effective regulators of osteoclast metabolism and bone homeostasis, and played a critical role in the prevention and treatment of metabolic syndrome. The treatment of Puerarin modulated the GM disorder to elicit the anti-OP effects in the OVX-induced rats by improving the bone micro-environment via regulating the levels of short-chain fatty acids (SCFAs) and repairing the intestinal mucosal integrity. In addition, Li et al. [9] indicated that tuna bone powder (TBP) stimulated bone formation via Wnt/ $\beta$ -catenin pathway activation and inhibited bone resorption via NF- $\kappa$ B pathway suppression to alleviate the GIOP. Meanwhile, GC significantly increased the transcription levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1, IL-6, and IL-17 in colon, whereas TBP treatment at high-dosage significantly decreased the transcription of all the above inflammatory cytokines, thus relieving systemic inflammation and intestinal integrity. Cheng et al. [32] showed that long-term alcohol consumption induced OP and affected the composition of GM, and alcohol can activate T lymphocytes directly or indirectly regulate the alterations of GM to produce the cytokines, and further activate osteoclasts. Moreover, the status of OP was more severe in old rats than young rats, which may be due to the higher diversity and stronger regulation ability of GM in young rats compared with old rats. Li et al. [48] revealed that the acceleration of senescence caused by the administration of D-galactosamine (D-gal) and NaNO<sub>2</sub> to mice showed an osteoporotic bone phenotype, which was related to aggravated oxidative stress, activated Sirt6/NF- $\kappa$ B/CatK signaling, and disruption of GM. Remarkably, *Fructus ligustri lucidi* (FLL) aqueous extract might preserve the aging-related bone quality via inhibiting the above-mentioned signaling pathways [48]. In addition to this, Wang et al. [49] suggested that the feces from senile osteoporotic rats transferred to young rats could induce senile OP, indicating altered GM and impaired intestinal barrier contributed to the pathogenesis of OP. Zhang et al. [50] showed that fecal microbiota transplantation (FMT) ameliorated the bone loss in mice with OVX-induced OP through modulating the GM and metabolic function. To be specific,

FMT prevented the OVX-induced bone loss by correcting the imbalance of GM, improving the level of SCFAs, optimizing the intestinal permeability, and inhibiting the release of pro-osteoclastogenic cytokines, which might be an option to serve as a promising candidate for the prevention and treatment of OP in the future.

In general, GM was closely associated with OP (the animals' studies were shown in Table 1, and the population-based researches are shown in Table 2) [51–63]. Recent studies have underscored the emerging role of GM in regulating bone metabolism. The modification of GM could be a viable therapeutic strategy to regulate the bone metabolism under a variety of conditions that lead to bone loss and OP. Nonetheless, relevant studies on the relationship between GM and OP are still in the initial stage, and more researches are required to further clarify its in-depth mechanisms and explore effective treatments with less side effects.

## Relevant mechanisms of regulating OP through GM

### Inflammatory regulation

The regulatory role of inflammatory mediators in bone metabolism has been well explored [64]. In addition to osteoclasts and osteoblasts, T cells, B cells, hemopoietic cells, and osteocytes could play a role in inflammatory regulation and bone loss, which have also been shown to be the generators of RANKL [65]. Osteoclast-induced bone resorption is activated by oxidative and inflammatory cytokines such as nitric oxide (NO), IL-1 $\beta$ , IL-6, IL-8, IL-18, IL-15, IL-17, IL-32, and TNF- $\alpha$ , many of which induce the osteoclastogenesis by upregulating the release of RANKL [66]. TNF- $\alpha$  inhibits the mesenchymal stromal cells (MSCs) to the transition of osteoblasts by regulating the expression of recombinant runt related transcription factor 2 (RUNX2), a master transcription factor that commits MSCs into osteogenic pathway. TNF- $\alpha$  targets the expression of Osterix, a key transcription necessary for osteoblast maturation [67, 68]. By contrast, bone resorption is down-regulated by anti-inflammatory cytokines, such as IL-3, IL-4, IL-10, IL-13, and transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) [69]. NO and anti-inflammatory cytokines affect the osteoblasts by increasing osteoprotegerin (OPG) and decreasing the production of RANKL, thus generating a high OPG to RANKL ratio that favors the inhibition of osteoclast differentiation [66]. Lipopolysaccharides (LPS), the important component of lipoproteins derived from gram-negative GM, is a strong stimulatory endotoxin triggering the inflammatory immune responses and influencing the health, which escalates the oxidative stress and inflammation, activating osteoclasts and bone resorption. In osteoblasts, LPS activates the NF- $\kappa$ B

**Table 1** Researches regarding the GM and OP at animals' level

Researchers	Sample	n	Group	Methods	Results	Conclusion/significance
Li et al. [34]	Male ICR mice	40	(i) Normal <i>n</i> = 10 (ii) Aging <i>n</i> = 10 (iii) Aging + VE <i>n</i> = 10 (iv) Aging + FLL <i>n</i> = 10	Normal: 0.9% sterile saline Aging: D-gal (120 mg/kg/d) + NaNO <sub>2</sub> (90 mg/kg/d) Aging + VE: D-gal + NaNO <sub>2</sub> + VE (100 mg/kg/d; dissolved in 0.1% Tween 80) Aging + FLL: D-gal + NaNO <sub>2</sub> + FLL (4.9 g/kg/d; dissolved in 0.1% Tween 80)	FLL modulated GM composition in aging mice	The acceleration of senescence caused by administration of D-gal and NaNO <sub>2</sub> to ICR mice exhibited an osteoporotic bone phenotype. This was associated with aggravated oxidative stress and activated Sirt6/NF-κB/CatK signaling and the disruption of GM, including increased levels of FMO3 and TMAO
Li et al. [9]	Female SD rats	40	(i) Sham <i>n</i> = 10 (ii) OVX <i>n</i> = 10 (iii) OVX + P(L) <i>n</i> = 10 (iv) OVX + P(H) <i>n</i> = 10	OVX + P(L): OVX + puerarin (50 mg/kg/d) OVX + P(H): OVX + puerarin (100 mg/kg/d)	Puerarin regulated the imbalance of GM in OVX rats	Puerarin treatment improved the bone microenvironment to inhibit OVX-induced OP via modulating SCFAs released by the GM and repairing intestinal mucosal integrity
Li et al. [23]	ICR mice	48	(i) Control <i>n</i> = 8 (ii) Model <i>n</i> = 8 (iii) CC <i>n</i> = 8 (iv) LD <i>n</i> = 8 (v) MD <i>n</i> = 8 (vi) HD <i>n</i> = 8	Control: 0.9% sterile saline CC: CaCO <sub>3</sub> (570 mg/kg/d) LD: GC + TBP (500 mg/kg/d) MD: GC + TBP (1000 mg/kg/d) HD: GC + TBP (1500 mg/kg/d)	TBP modulates the GM	TBP exerted anti-osteoporosis effects in GIOP mice. Beneficial effects also result from GM modulation, with enhanced SCFAs productions and anti-inflammatory bacterial abundance
Schepper et al. [10]	Male C57BL/6 J mice	39	(i) Control <i>n</i> = 10 (ii) GC <i>n</i> = 9 (iii) ABX <i>n</i> = 10 (iv) ABX + GC <i>n</i> = 10	GC: 2.5 mg/kg/d ABX: ampicillin and neomycin, 160 and 80 mg/kg/day	GC treatment alters fecal microbiota composition	Depletion of the GM prevents GC-induced trabecular bone loss
Liu et al. [2]	Female SD rats	40	(i) Control <i>n</i> = 8 (ii) GC <i>n</i> = 8 (iii) L <i>n</i> = 8 (iv) M <i>n</i> = 8 (v) H <i>n</i> = 8	Control: saline (twice a week, 8 weeks) GC: dexamethasone (0.1 mg/100 g twice a week, 8 weeks) L: GC + APS (50 mg/kg) M: GC + APS (150 mg/kg) H: GC + APS (250 mg/kg)	APS improved GC-induced OP in rats	Our studies discovered the structural and functional changes of GM in APS-improved OP, and inferred the potential key bacteria
Shen et al. [28]	Female SD rats	40	(i) Control <i>n</i> = 8 (ii) OVX <i>n</i> = 8 (iii) OVX-E <i>n</i> = 8 (iv) OVX-CS <i>n</i> = 8 (v) OVX-CSCa <i>n</i> = 8	OVX: saline OVX-E: 17β-estradiol (100 µg/kg) OVX-CS: CS (150 mg/kg) OVX-CSCa: CSCa (150 mg/kg)	GM structure change caused by CSCa intervention	CSCa, instead of CS, dietary intervention can alleviate the estrogen deficiency-induced OP, which probably involves the GM and metabolite profiles change in the faeces
Wang et al. [132]	Female SD rats	32	(i) Control <i>n</i> = 16 (ii) FMT <i>n</i> = 16	Control: sterile saline oral gavage of 1 ml/rat three times a week FMT: fresh fecal samples of four 18-month-old female SD rats oral gavage of 1 ml/rat three times a week	GM from senile rats increased bone turnover in 3-month-old rats	GM from senile osteoporotic rats could induce OP in young rats

**Table 1** (continued)

Researchers	Sample	n	Group	Methods	Results	Conclusion/significance
Ma et al. [54]	Female SD rats	8	(i) Control <i>n</i> = 4 (ii) OP <i>n</i> = 4	Control: 22-month-old rats OP: 6-month-old rats	There was a significant reduction in alpha diversity and the F/B ratio in aged rats	Shifts in the GM contribute to senile osteoporosis through metabolic pathways and subsequent immune disorders
Tao et al. [63]	Female C57BL/6 mice	28	(i) Sham <i>n</i> = 7 (ii) OVX <i>n</i> = 7 (iii) Low-UA <i>n</i> = 7 (iv) High-UA <i>n</i> = 7	Sham: 0.5% CMC-Na treatment OVX: OVX + 0.5% CMC-Na Low-UA: OVX + 10 mg/kg UA High-UA: OVX + 20 mg/kg UA	UA reduced OVX-induced bone loss in mice	UA, a metabolite of the GM, ameliorated OVX-induced bone loss by suppressing osteoclast formation
Lan et al. [55]	Male C57BL/6 J mice	40	(i) BC <i>n</i> = 8 (ii) MC <i>n</i> = 8 (iii) H <i>n</i> = 8 (iv) M <i>n</i> = 8 (v) L <i>n</i> = 8	BC: 0.9% NaCl MC: 0.9% NaCl + 2% DSS (1 week) + 1% DSS (1 week) H: BL-99 ( $1 \times 10^{11}$ CFU/d/mouse) + 2% DSS (1 week) + 1% DSS (1 week) M: BL-99 ( $1 \times 10^9$ CFU/d/mouse) + 2% DSS (1 week) + 1% DSS (1 week) L: low-dose BL-99 ( $1 \times 10^7$ CFU/d/mouse) + 2% DSS (1 week) + 1% DSS (1 week)	BL-99 administration is associated with changes in the composition of the GM	BL-99 administration was sufficient to prevent and alleviate DSS colitis-induced bone loss-associated inflammation and associated bone loss in mice
Cheng et al. [32]	SD rats	12	(i) Control <i>n</i> = 6 (ii) Alcohol <i>n</i> = 6	Control: isocaloric liquid diet with maltose-dextran substituted for ethanol Alcohol: Bio-Serv Liquid Rat Diet LD82 containing 5.0% alcohol (35% alcohol-derived calories) which the dietary concentration of ethanol was increased from 0 to 35%	A diversity of fecal bacterial flora in young and old rats increased significantly after alcohol intake	Long-term alcohol consumption could affect the expression of bone metabolism regulating hormones, induce the activation of osteoclast differentiation and influence the composition of GM in rats
Liu et al. [19]	Male SD rats	27	(i) Saline <i>n</i> = 9 (ii) Ethanol <i>n</i> = 9 (iii) Antibiotic <i>n</i> = 9	Saline: saline (10 ml/kg, 6 times/week, 1 time/d, 16 weeks) Ethanol: 20% ethanol (10 ml/kg, 6 times/week, 1 time/d, 16 weeks) Antibiotic: 1 g/L metronidazole and 0.2 g/L ciprofloxacin (10 ml/kg, 6 times/week, 1 time/d, 16 weeks)	Compared with antibiotics application, ethanol-gavaged decreased the BMD in rats	Chronic heavy ethanol consumption causes osteoporosis and GM dysbiosis in rats, indicating that the regulation of the gut-bone axis might contribute to the ethanol-induced bone loss



Table 1 (continued)

Researchers	Sample	n	Group	Methods	Results	Conclusion/significance
Wang et al. [132]	Female C57BL/6J mice	30	(i) Sham + Vehicle n = 10 (ii) OVX + Vehicle n = 10 (iii) OVX + P. histicola n = 10	Sham + Vehicle: plain culture medium OVX + Vehicle: plain culture medium OVX + P. histicola: 0.1 mL bacteria ( $1 \times 10^9$ CFU) every other day for 12 weeks	P. histicola-treated OVX mice maintained a relatively higher bone volume than OVX controls	P. histicola could prevent estrogen deficiency-induced bone loss through the GM-bone axis
He et al. [30]	Female NTac: SD rats	49	(i) DCa n = 7 (ii) DCa-La n = 7 (iii) DCa-In n = 7 (iv) CaC n = 7 (v) CaC-La n = 7 (vi) CaC-In n = 7 (vii) Control n = 7	CaC: OVX + Altromin C1031 added 0.5% calcium carbonate CaC-La: OVX + Altromin C1031 added 0.5% calcium carbonate + 0.5% lactose CaC-In: OVX + Altromin C1031 added 0.5% calcium carbonate + 5% inulin DCa: Altromin C1031 added 2% Capolac DCa-La: Altromin C1031 added 2% Capolac + 0.5% lactose DCa-In: Altromin C1031 added 2% Capolac + 5% inulin	Rats fed with calcium-fortified diets have higher BMD, bone mineral content and femur mechanical strength, lower serum levels of bone markers, and lower expression of calcium absorption-related genes TRPV6, CaBP compared with control	Calcium modulates GM composition and function
Liu et al. [51]	Male Wistar rats	18	(i) Control n = 6 (ii) Model n = 6 (iii) GSD n = 6	Control: saline Model: prednisolone (15 mg/kg twice a week) GSD: prednisolone (15 mg/kg twice a week) + GSD (30 g/kg/d) twice a week	GM composition might be modified	GSD could play an anti-osteoporosis role by regulating these metabolic pathways
Li et al. [65]	Female C57BL/6 J mice	20	(i) Sham n = 10 (ii) OVX n = 10	Sham: LGG or VSL#3 ( $1 \times 10^9$ CFU twice a week) OVX: OVX + LGG or VSL#3 ( $1 \times 10^9$ CFU twice a week)	Feeding of LGG or VSL#3 increased bone formation in ovx mice	Supplementation of the normal flora of sex steroid-depleted mice with the commonly used probiotics LGG or VSL#3 significantly tightened intestinal barrier integrity and completely protected mice against sex steroid depletion-induced bone loss

GM gut microbiota, OP osteoporosis, FLL *Fructus Ligustri Lucidi*, D-gal D-galactose, VE vitamin E, TMAO trimethylamine-N-oxide, NF- $\kappa$ B nuclear factor-kappa B, CatK cathepsin K, Sirt6 sirtuin 6, FMO3 flavin-containing monooxygenase 3, P(L) a low-dose puerarin, P(H) a high-dose puerarin, OVX ovariectomy, SCFAs short chain fatty acids, d day, SD Sprague-Dawley, TBP tuna bone powder, GIOP glucocorticoid-induced osteoporosis, CC CaCO<sub>3</sub>, LD low dose, MD medium dose, HD high dose, L low dose, M medium dose, H high dose, GCs glucocorticoids, ABX broad-spectrum antibiotics, APS astragalus polysaccharides, GS chondroitin sulfate, CSCa chondroitin sulfate calcium complex, E 17 $\beta$ -estradiol, FMT fecal microbiota transplantation, F/B firmicutes/bacteroidetes, UA Urolithin A, CMC-Na sodium carboxymethyl cellulose, BC blank control, MC model control, DSS dextran sodium sulfate, BMD bone mineral density, BL-99 *Bifidobacterium lactis* BL-99, TRPV6 transient receptor potential vanilloid type 6, CaBP calcium-binding protein, GSD gushudan

**Table 2** Researches regarding the GM and OP at population-based level

Researchers	Sample	n	Group	Methods	Results	Conclusion/significance
Takimoto et al. [57]	Postmenopausal Japanese women	61	(i) Placebo n = 30 (ii) C-3102 n = 31	C-3102: <i>Bacillus subtilis</i> C-3102 ( $3.4 \times 10^9$ CFU/d)	C-3102 treatment resulted in a significant change in the relative abundance of 11 bacterial genera	C-3102 modulates host GM and inhibits bone resorption
Jansson et al. [46]	Swedish healthy postmenopausal women	249	(i) Placebo n = 123 (ii) <i>Lactobacillus</i> n = 126	<i>Lactobacillus</i> : DSM 13,434 + DSM 15,312 + DSM 15,313 ( $1 \times 10^{10}$ CFU/capsule/d)	<i>Lactobacillus</i> treatment reduced the LS-BMD loss compared with placebo	Probiotic treatment using a mix of three <i>Lactobacillus</i> strains naturally occurring in the human GM protects against LS-BMD loss in healthy postmenopausal women
Nilsson et al. [62]	Postmenopausal women with osteopenia	90	(i) Placebo n = 45 (ii) LR6475	LR6475: <i>Lactobacillus reuteri</i> ATCC PTA6475 ( $1 \times 10^{10}$ CFU/d)	<i>L. reuteri</i> 6475 reduced the loss of total vBMD compared to placebo	Daily supplementation with <i>L. reuteri</i> 6475 for 12 months reduced loss of tibia total vBMD in older women with low BMD
Lambert et al. [56]	Postmenopausal osteopenic women	78	(i) Placebo n = 40 (ii) RCE n = 38	Placebo: placebo extract + CDM/d RCE: twice daily RCE ( $2 \times 95$ mL = 60 mg isoflavones/d total) + CDM/d	A significant reduction in bone resorption marker plasma CTx concentration was found in the RCE group	RCE attenuate the BMD loss and improve the bone turnover
Morato-Martínez et al. [47]	Postmenopausal women with osteopenia	78	(i) CG n = 39 (ii) EG n = 39	CG: a daily ration of the same product but without enrichment EG: one serving a day of the experimental product enriched with bioactive bone nutrients	Consumption of an experimental dairy product resulted in a significant improvement in bone mass content and managed to mitigate the loss of BMD, and the intake of calcium and vitamin D improved significantly	Consumption of functional foods with a milk matrix enriched in nutrients essential for bone health could contribute to the primary prevention of OP and thus decrease the morbid mortality rates and the costs associated with the disease
Jafarnejad et al. [58]	Postmenopausal women with osteopenia	50	(i) Probiotic n = 25 (ii) Control n = 25	Probiotic: gerilact capsules + 500 mg Ca + 200 IU vitamin D/d Control: placebo capsules + 500 mg Ca + 200 IU vitamin D/d	Serum TNF- $\alpha$ , as a major pro-inflammatory cytokine, decreased significantly in the probiotic group compared to the placebo group	Multispecies probiotic supplementation among postmenopausal osteopenic women showed a possible role of OC, BALP, PTH, and TNF- $\alpha$ in suppressing bone resorption and bone turnover
Yang et al. [45]	Postmenopausal Chinese women with osteoporosis or osteopenia	132	(i) OP (n = 34) (ii) OPe (n = 47) (iii) Control (n = 51)	Enzyme-linked immunosorbent assay: The serum levels of IL-10, TNF- $\alpha$ , and LBP 16S rRNA gene V3-V4 region sequencing was performed to investigate the GM and VM	It was noted that for GM, <i>Romboutsia</i> , unclassified_Mollicutes, and <i>Weissella</i> spp. were enriched in the control group, whereas the abundances of Fusicatenibacter, <i>Lachnospiridium</i> , and <i>Megamonas</i> spp. were higher in the OP group	The results show that changes in BMD in postmenopausal women are associated with the changes in GM

Table 2 (continued)

Researchers	Sample	n	Group	Methods	Results	Conclusion/significance
Ozaki et al. (2020)	Postmenopausal Japanese women	38	(i) high BMD $n=19$ (ii) low BMD $n=19$ Or (i) high vitamin K2 $n=10$ (ii) low vitamin K2 $n=28$	GM profiling: 16S rRNA gene sequencing Serum bone turnover markers: vitamin K fraction and TRACP-5b BMD: forearm dualenergy X-ray absorptiometry	Rikenellaceae was significantly abundant in the low BMD group. Bacteroides and Sutterella were predominant in the high vitamin K2 group	Bacteroides and Rikenellaceae may be involved in bone metabolism and fracture risk
Das et al. [35]	Adult female and male subjects from Ireland	181	(i) OPe $n=61$ (ii) OP $n=60$ (iii) normal BMD $n=60$	Analysis of the 16S (V3-V4 region) amplicon dataset classified to the genus level was used to identify significantly differentially abundant taxa	A detailed study of microbiota associations with meta-data variables that included BMI, health status, diet and medication revealed that these meta-data explained 15–17% of the variance within the microbiota dataset	Reduced BMD in OPe and OP is associated with an altered microbiota
Xu et al. [59]	Chinese orthopedic inpatients	96	(i) PO $n=48$ (ii) Control $n=48$	PO: Primary Osteoporosis patients Control: Healthy patients 16 s rDNA amplification sequencing	The diversity of GM in PO patients was significantly higher than that in control group and there was significant difference in microbial composition in PO group	PO is related to the change of GM, especially the enriched Dialister and Faecalibacterium genera
Li et al. [34]	Healthy Chinese adults	102	(i) Control $n=51$ (ii) Low-BMD $n=51$	16S rRNA gene sequencing	The low-BMD individuals had a smaller number of OTUs and bacterial taxa at each level	Several taxa with altered abundance and specific functional pathways were discovered in low-BMD individuals
Wei et al. [61]	Han Chinese adults older than 60 years or postmenopausal women	108	(i) Control $n=64$ (ii) OP $n=44$	16S rRNA gene sequencing	Patients with OP had higher absolute and relative abundances of Bacteroidetes phylum, and Bacteroides and Eisenbergiella genera	GM compositions may contribute to the risk of OP
He et al. [30]	Postmenopausal Chinese women	106	(i) OPe $n=33$ (ii) OP $n=42$ (iii) normal BMD $n=31$	16S rRNA gene sequencing LC-MS-based metabolomics approach	Decreased bacterial richness and diversity in PMOP	We described the disordered profiles of GM and fecal metabolomes in postmenopausal women with OPe and OP
Wang et al. [132]	Postmenopausal Chinese women	42	(i) PMO $n=24$ (ii) PMN $n=18$	16S rRNA gene sequencing	GM in the PMO group featured a significantly decreased proportion of the genus Prevotella in comparison with that in the PMN group	The proportion of the genus Prevotella was notably higher in the PMN group, demonstrating its potential bone-protective effects on osteoporosis

GM gut microbiota, OP osteoporosis, LS-BMD lumbar spine bone mineral density, C-3102 *Bacillus subtilis* C-3102, DSM 13434 *L. paracasei* 8700-2, DSM 15312 *L. plantarum* Heal 9, DSM 15313 *L. plantarum* Heal 19, BMD bone mineral density, vBMD volumetric bone mineral density, L. reuteri 6475 *Lactobacillus reuteri* ATCCPTA 6475, RCE red clover extract, CDM vitamin and mineral tablets containing 1040 mg Ca, 487 mg Mg and 25 mg vitamin D, CG control group, EG experimental group, TRACP-5b tartrate-resistant acid phosphatase 5b, IL-10 interleukin-10, TNF- $\alpha$  tumor necrosis factor- $\alpha$ , LBP lipopolysaccharide-binding protein, BMI body mass index, PO primary osteoporosis, VM vaginal microbiota, PMOP postmenopausal osteoporosis, PMO postmenopausal women with osteoporosis, PMN postmenopausal women with normal bone mass, LC-MS-based liquid chromatography-mass spectrometry based



and inducible nitric oxide synthase (iNOS) [69, 70]. GM also promotes the endothelial NOS (eNOS) and increased the inflammatory cytokines, including the TNF- $\alpha$ , IL-1, and IL-6, repressing trabecular bone mineralization [69]. The estrogen-deficiency decreases intestinal permeability and represses estrogen receptor-mediated signaling pathways, such as GTP-binding protein Ras, Raf, IFN- $\gamma$ , and mitogen-activated protein kinase (MAPK) in intestinal epithelial microenvironment [71]. Toll-like receptors (TLRs) play pivotal roles in inflammation and provide vital links between the immune and skeletal systems. According to Ding et al. [72], TLR9-deficiency causes osteoclastic bone loss via altering the composition of GM and inducing systemic chronic inflammation. In detail, *Mucispirillum schaedleri* and *Parabacteroides distasonis* presented significant enhancement in the TLR9<sup>-/-</sup> mice. GM could affect the inflammation state to regulate the bone mass, and the inflammation is also closely associated with bone metabolism [73]. Tousein et al. [74] observed that treatment with resistant starch can attenuated OVX-induced bone loss, which enhanced the abundance of *Bifidobacterium* in the feces, up-regulated the expression of anti-inflammatory cytokines in the colon, and reduced the expression of osteoclastogenic cytokines in the bone marrow of OVX-induced mice. Using the tilapia nilotica head lipids (THLs) on OVX-induced rats, Zhu et al. [75] demonstrated that the levels of pro-inflammatory cytokines had decreased notably after the intervention of THLs, which reduced the inflammation and prevented the bone resorption. Long-term high fat diet (HFD) resulted in the decreased bone mass, combined with GM dysbiosis, leaky gut, and systemic inflammation. Zhang et al. [76] revealed that the *Fructooligosaccharides* (FOS) and/or *galactooligosaccharides* (GOS) reversed high intestinal permeability and pro-inflammatory cytokines, alleviated intestinal and systemic low-grade inflammation induced by HFD, thus protecting against HFD-induced OP.

## Immune regulation

A critical role of immune system is to facilitate the beneficial effects of symbiotic relationship while simultaneously preventing the invasion of the host by pathogenic organisms in GM [29]. It is currently clear that the interactions of immune system with GM have profound effects on the bone health [77, 78]. GM contributes to the maturation of the immune system in early life [77]. Villa et al. [79] reviewed that in the absence of the GM, the mucosal immune system was characterized by hypoplastic Peyer's patches containing minimal germinal centers and a reduced number of IgA-producing plasma cells and lamina propria CD4 + T cells. In addition, the absence of GM results in immature systemic immunity with fewer and smaller germinal centers and reduced the number of CD4 + T cells

in the spleen. GM modulates immune system through the production of molecules with immunomodulatory and anti-inflammatory function that are capable to influence the immune cells [77]. RANKL is considered as main cytokine in osteoclast differentiation which is produced by mesenchymal cells, osteoblasts, and osteocytes in the bone marrow [27]. During the process of inflammation, activated CD4 + T cells are also a source of RANKL, as well as other cytokines including IL-17 and TNF- $\alpha$ , which suppress the differentiation of MSCs into osteoblasts and stimulate osteoclastogenesis [80, 81]. Moreover, the relationship between GM and bone is also mediated by innate immunity through several receptors such as nucleotide-binding oligomerization domain proteins (NOD1 and NOD2) receptors and TLR5 [82]. NOD1 and NOD2 are ubiquitarily intracellular sensors of the pathogen-associated molecular patterns (PAMPs), mainly expressed on the epithelial and immune cells, that bind bacterial peptidoglycans and activate the NF- $\kappa$ B pathway playing a key role in the effects of microbiota on bone [83]. The effects of GM on host immunity can be modulated by targeting Th17 cells and/or Tregs [84]. For instance, T-cell antigen receptor (TCR), specifically expressed by Th17 cells, could respond to antigens encoded by symbiotic segmented filamentous bacteria (SFB) [85]. SFB-mediated activation of TCR can induce Th17 cell expansion, which negatively affects skeletal maturation [86]. Moreover, *Bifidobacteria* and *Streptococcus thermophilus* can increase the concentration of TGF- $\beta$ , regulate the differentiation of Tregs/Th17 cells, thereby indirectly regulating the immune responses [87]. Gut-derived bacterial metabolites regulate the distant organs, among which indole derivatives were among the first bacterial metabolites to be described to affect the intestinal immunity [88, 89]. The indoles and indole derivatives, such as indole-3-aldehyde (IAld), indole-3-acetic-acid (IAA), indole-3-propionic acid (IPA), indole-3-acetaldehyde (IAAld), and indoleacrylic acid, are the bioactive substances produced directly through the activity of tryptophanase in *Escherichia coli* and *Lactobacillus spp* [90]. In particular, SCFAs, the family of metabolites produced by GM, that has received the greatest attention for its capacity to diffuse to the distant organs and induce potent regulatory effects, which recently have been recognized as the pivotal regulators of bone resorption and bone formation [91]. SCFAs blunt the osteoclast differentiation, and the inhibition of histone deacetylase (HDAC) activity is one of the mechanisms whereby this occurs [92]. It has been verified that increased solubility of minerals caused by enriched SCFAs, promoted absorption surface, and enhanced expression of calcium-binding proteins are the underlying mechanisms of probiotics or prebiotics facilitating mineral utilization [93].

## Endocrine regulation

Endocrine regulators of the energy metabolism, such as insulin and leptin, might influence the bone metabolism by changing substrate availability, interaction with bone cells or indirect signaling through central nervous system. Insulin-like growth factor 1 (IGF-1), a growth factor known to impact bone via endocrine and paracrine–autocrine mechanisms, also needs to be considered as a possible pathway for microbial impacts on bone [94]. Yan et al. [95] demonstrated that levels of IGF-1, a growth factor known to regulate skeletal formation, are dynamically modulated by changes in GM, thus modulating the anabolic stimulus to the skeleton. IGF-1 increases longitudinal femur development, and cartilage-specific deletion of receptor reveals that IGF-1 is required for growth plate maturation and secondary ossification center development [96]. Mice with a defined microbiota had greater IGF-1 levels than germ-free (GF) mice, indicating that GM can influence the levels of IGF-1, and IGF-1 has a significant impact on bone development and healthy maintenance [97, 98]. Reid et al. [99] revealed that leptin was a key regulator of energy intake and had also been shown to regulate bone metabolism and bone mass in the rodents and humans. Leptin receptor expression decreases as osteoblast differentiation advances, and leptin has a direct anabolic effect on the cells of osteoblast lineage. Moreover, leptin inhibition of osteoclast generation in the cultures of human peripheral blood mononuclear cells, and leptin increased the expression of OPG in cultures, suggesting that the inhibitory effect was mediated via the RANK/OPG system [100]. The endocrine regulation of bone cell activity, including that of leptin and insulin, might act as a sensor of substrate availability needed for the bone metabolism, suggesting that other endocrine factors related to energy availability may be involved in the regulation of bone cell activity [101]. Parathyroid hormone (PTH) is a calcitropic hormone critical for skeletal development. Similar to the butyrate, PTH stimulates the bone formation and induces the bone anabolism via Treg/Wnt10b/Wnt signaling pathway [102]. Using GM depletion by wide-spectrum antibiotics and GF female mice, Li et al. [65] showed that GM was required for PTH to stimulate bone formation and increase bone mass. GM depletion lowered the level of butyrate, a metabolite responsible for gut–bone communication, while the reestablishment of physiologic levels of butyrate restored PTH-induced anabolism [103]. Estrogen depletion observed in post-menopausal women adversely impacts the bone homeostasis, and one of the principal regulators of circulating estrogens is GM [104]. GM regulates estrogens through the secretion of  $\beta$ -glucuronidase, an enzyme that deconjugates estrogens into their active forms. When this process is impaired through, for example, lower diversity of

GM, the decrease in deconjugation results in a reduction of circulating estrogens [105]. Excessive osteoclast formation and resorption are considered as the key pathological alterations in estrogen-deficiency induced OP [106]. Estrogen deprivation also increases intestinal permeability allowing the translocation of bacteria and increasing the number of antigens entering the epithelial mucosa what could lead to systemic inflammation [107]. In summary, there is a complicated and close link between GM and OP, and overall mechanisms of three patterns of regulation (inflammation, immune, endocrine) are shown in Fig. 1.

## The effects of GC on GM

### The effects on intestinal mucosal barrier

The gastrointestinal tract is the largest surface facing outside environment, being in direct contact with the commensal microbiota and antigens from the diets [108]. To ensure the homeostasis, the intestine acts as a permeable but selective barrier, absorbing nutrients and water to obtain energy and blocking the passage of antigens and bacteria to inner milieu. This critical property of the gut is known as intestinal barrier function (IBF). The regulation of paracellular permeability is exerted chiefly at the tight junction level [109]. The tight junctions are constituted by the transmembrane proteins (claudins, occludins, tricellulin and the junctional adhesion molecule) and peripheral membrane associated proteins that connect to the actin cytoskeleton (zonulae occludens (ZO) 1–3, AF-6, and cingulin) [110]. In most cases, the pathologically increased permeability, for instance, by dysregulated secretion of pro-inflammatory cytokines is associated with the modulation of tight junctions [111]. GC increased the intestinal permeability (barrier leaks) as evidenced by increased levels of endotoxin in the serum. More importantly, the treatment of high-molecular-weight polymer effectively prevented the GC-induced elevation of serum endotoxin and correspondingly prevented femoral trabecular bone loss induced by GC. *Lactobacillus reuteri* and chronic antibiotic treatments prevented GC-induced barrier leaks. Together, GC-altered barrier dysfunction is a key pathogenic event in GIOP [112].

### The effects on GM-related metabolites

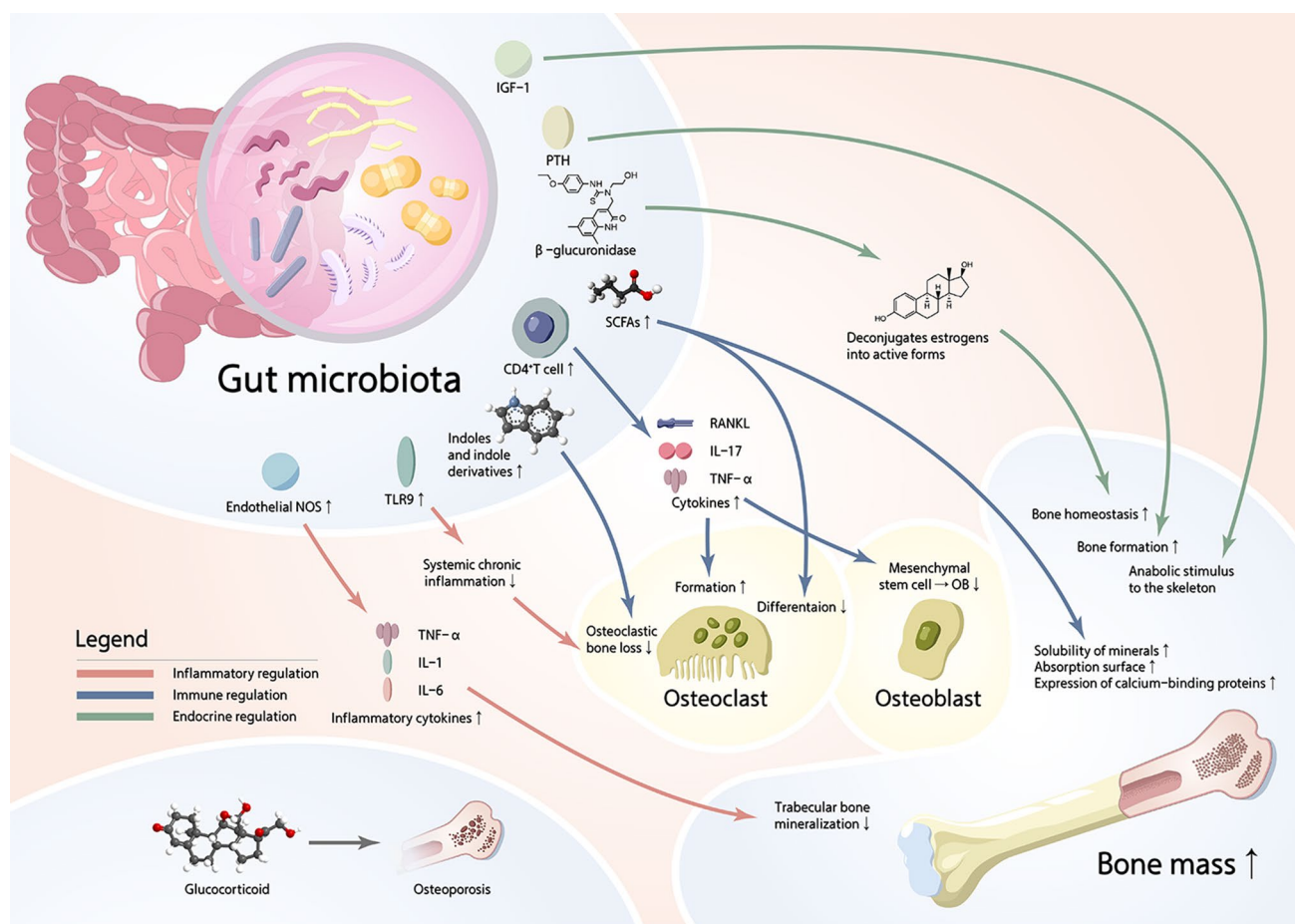
In this term, Qiu et al. [113] used gas chromatography to measure levels of SCFAs from 55 individuals, including patients with GC-induced obesity, and age- and gender-matched healthy controls. The result revealed that the overall content of SCFAs in GCs-induced obesity group tended to be lower than that of healthy control. Correspondingly, they observed that propionate and butyrate also decreased

dramatically in that group. Zhang et al. [114] revealed that the treatment of prednisone altered the profile of fecal metabolites in the rats, and the changed fecal metabolites included SCFAs, fatty acids, amino acids, organic acids, benzenoids, and phenylpropanoic acids. Collectively, 11 down-regulated and 10 up-regulated metabolites were identified based on a volcano plot and a variable importance in projection analysis. In details, decreased metabolites included valeric acid, propanoic acid, isobutyric acid, isovaleric acid, caproic acid, hydrocinnamic acid, 2-phenylpropionate, phenylacetic acid, orthohydroxyphenylacetic acid, acetoacetic acid, and ethylmethylacetic acid. On the contrary, phenyllactic acid (PLA), hydroxyphenyllactic acid (OH-PLA), homovanillic acid, m-aminobenzoic acid, malonic acid, succinic acid, methylmalonic acid, 2-hydroxy-3-methylbutyric acid, L-tryptophan, and L-phenylalanine (PAH) increased significantly. Moreover, the pathway analysis suggested that differential metabolites were enriched in pathways, including the phenylalanine metabolism, butanoate metabolism, and propanoate metabolism. In the survey conducted by Marazzato et al. [115], the metabolomic analysis of SCFAs showed that early

rheumatoid arthritis (ERA) patients presented significantly decreased propanoic acid levels at baseline compared to controls. Such differences persisted after standard administration (methotrexate plus GC) to the ERA group but the tendency to increase.

### The effects of oxidative stress

Oxidative stress, which is induced by the excessive reactive oxygen species (ROS) production and/or an impaired antioxidant system, has been revealed as an underlying mechanism for loss of bone mass and quality [116]. ROS includes the superoxide anion ( $O_2^-$ ), hydroxyl ( $HO^\bullet$ ), singlet oxygen ( $^1O_2$ ), and hydrogen peroxide ( $H_2O_2$ ) [117]. They are highly reactive molecules formed upon incomplete reduction of oxygen during the aerobic metabolism [118]. GC has been reported to induce ROS generation, activating the PKC $\beta$ /p66shc/JNK signaling cascade, leading to apoptosis. In response to ROS, the activity of forkhead box O (FoxO) is spontaneously enhanced, along with inhibited Akt phosphorylation and attenuated osteoblastogenesis [119].



**Fig. 1** The overall mechanisms of the patterns of regulation (inflammation, immune, endocrine)

Supraphysiologic GC disturbs the balance between free radical generation and the scavenging activities of intracellular antioxidants, which results in oxidative stress [44]. There is currently enough evidence that oxidative stress and the development of OP go hand in hand. Excessive GC induces oxidative stress due to the production of enormous oxidants, leading to the apoptosis of osteoblasts [120]. The upregulation of GC could physiologically indicate increased stress levels, which may alter bone homeostasis, which induces osteoblast injury by triggering JNK phosphorylation mediated the production of ROS [121]. Overproduction of ROS also promotes lipid peroxidation leading to bone loss by inhibiting bone formation [122]. Rai et al. [121] delineated that compound 5e (a BMP2 secretagogue) activated the NRF2 signaling to counter the disturbed cellular redox homeostasis and escalate the osteoblast survival. On the other hand, 5e increased ALP, mineralization activity, and promoted osteoblast differentiation by activating WNT/ $\beta$ -catenin signaling in BMP2-dependent manner, which ameliorated the GC-induced oxidative stress in osteoblasts. Hua et al. [119] reviewed that connexin hemichannel opening was increased under oxidative stress conditions, which conferred a cell protective role against the oxidative stress-induced cell death. Oxidative stress acts as a key contributor to the GIOP, and impairs osteocytic network and connexin gap junction communication [123, 124]. Lee et al. [125] reported that the elevated levels of oxidative stress may increase bone resorption by promoting osteoclastogenesis and inhibiting osteogenesis and ginkgolide B (GB), a small natural molecule from ginkgo biloba, possesses pharmacological activities by regulating the ROS in GIOP. In female mice with GIOP models, the oral gavage of GB significantly improved bone mass consistent with the increase in the OPG-to-RANKL ratio.

### Potential approaches for prevention and treatment of GIOP from perspectives of GM and its metabolites

Recent researches underscore the emerging role of the GM and metabolites in the treatment of OP [126, 127]. Warmth enhances bacterial polyamine biosynthesis, resulting in higher total polyamine levels in vivo and spermidine supplementation can increase bone strength. The warmth exposure (34 °C) protects against OVX-induced bone loss by increasing the trabecular bone volume, connectivity density, and thickness, leading to improved biomechanical bone strength in adult female, as well as in the young male mice [1]. Probiotics, which are microorganisms conferring a health benefit to the host when administered in adequate amounts, are present in fermented dairy products and some

vegetable-derived food such as sauerkraut and kimchi [128, 129]. Modification of the GM by ingesting probiotics could be a viable therapeutic strategy to regulate bone metabolism under various conditions that result in the bone loss and OP [130, 131]. *Prevotella histicola* could prevent estrogen deficiency-induced bone loss via the GM-bone axis in postmenopausal women, which may serve as a therapeutic agent or target for OP [132]. The intervention of dietary chondroitin sulfate calcium complex (CSCa) has potentials to alleviate the OP and OP-related symptoms probably involving GM or metabolite profiles as demonstrated in rats [28]. Arecanut seed polyphenol-ameliorated OP by altering GM via lysozyme and immune system in estrogen deficiency rats [133]. FMT, which refers to the transplantation of GM from healthy donors to recipients with GM imbalance, so that the GM in recipients can be reshaped and play a normal function, and further prevent or treat the diseases related to the GM disorder. As a novel “organ transplantation” technique, it can be as a promising treatment option for the OP [50, 88]. Besides, exercise could enrich the abundance and diversity of GM, improve proportion of *Firmicutes* and *Bacteroidetes*, induce the proliferation of beneficial microbiota, and improve the function of intestinal mucosal barrier, thereby further modulating the bone metabolism [83, 134, 135].

Currently, the individuals are trying to treat GIOP from the perspectives of GM and its metabolites. Current therapies for OP treatment by inducing osteoblast activity (such as parathyroid hormone treatment), or inhibiting osteoclast function (such as treatment of bisphosphonates), have certain limitations [136, 137]. Due to complex compositions of bioactive substances in natural products and its possible synergistic effects, natural product-derived alternatives for OP therapy are becoming more and more popular. Li et al. [9] reported that TBP alleviates GIOP via the coregulation of NF- $\kappa$ B and Wnt/ $\beta$ -Catenin signaling pathways and the modulation of composition of GM and metabolism, enhancing the abundance of anti-inflammatory bacteria and SCFAs producers. The GC treatment increased the abundance of *Bacteroidetes* and *Proteobacteria* and decreased *Firmicutes*, whereas TBP aggravated the alterations in *Bacilli* and *Clostridia* induced by GC and restored the abundance of *Actinobacteria*. In terms of effects of TBP on the production of SCFAs in feces, the contents of acetic, propionic, and n-butyric acids in feces were reduced in the model group and increased dose dependently after treatments with different dosages of TBP. Liu et al. [2] investigated APS-modified GM and the potential key bacteria to alleviate OP, as well as its relationship with improved OP. They demonstrated the anti-osteoporotic function of APS with restored BMD and repaired bone microarchitecture in a dexamethasone-induced OP rat model. In APS-treated rats, analysis on the bacterial community revealed that the structure of GM was



dramatically altered by APS, and the bacteria (*c\_Bacteroidia*, *p\_Bacteroidetes*, and *g\_Allprevotella*) could serve as biomarkers for APS-improved OP. In addition to this result, five genera (*uncultured\_bacterium\_f\_Ruminococcaceae*, *Alloprevotella*, *Ruminococcaceae\_UCG-014*, *Blautia* and *Lactobacillus*) were inferred as the key bacteria in APS-improved OP. Researches of GM change accordingly in various food and nutritional conditions which provide a basic knowledge for the future investigations of how interactions between the food components and GM may influence or even determine human health and disease [138–140]. Accordingly, Yun et al. [141] reported that bovine colostrum-derived exosomes (BCE) can delay the progress of GIOP and change the GM in GIOP mice. Osteoporotic mice fed a high BCE exhibited higher BMD and the percent of bone volume than the osteoporotic mice not fed BCE, indicating that BCE ingestion significantly prevented GIOP. Based on the heat map, the relative abundance of *Lactobacillus* and *Bacteroides* in PDS group was decreased more than that in the Sham group, but that decrease was significantly reversed by the intake of BCE. Moreover, GIOP increased *Firmicutes* and *Deferribacteres*, whereas *Bacteroidetes* were decreased. The increased *Deferribacteres* and *Bacteroidetes* in PDS group was significantly decreased by BCE. Schepper et al. [10] conducted an experiment with adult C57BL/6 J male mice, revealing that probiotic *Lactobacillus reuteri* 6475 supplementation prevents GC-induced bone loss. Moreover, the composition of GM was significantly different between the groups when examined at the level of OTUs, and the GC-induced bone loss was transferable by FMT. Wang et al. [142] presented that GM diversely changed in GIOP rats and Daphnetin treatment ameliorated this disorder effectively. PCoA plot indicated that the main components of GM in DEX group differed from the control group, while H-Daph treatment narrowed the difference between the GIOP rats and control rats. In addition, the expressions of *Clostridiales\_vadin\_BB60\_group* and *Peptostreptococcaceae* were all up-regulated, while the expression of *Bacteroidales\_RF16\_group* was down-regulated in GIOP rats, while the treatment of H-Daph restored the expressions of these bacterium. Pan et al. [143] systematically analyzed metabonomic characteristics of GIOP rats and elucidated the therapeutic effects of Epimedium (a common kind of traditional Chinese medicine), using a <sup>1</sup>H NMR-based metabonomic approach in conjunction with multivariate data analysis. They observed that GC led to the metabolic disorders of GM and an increase on phenylalanine. Compared with GIOP rats, the concentration of phenylacetyl glycine (a metabolic product of phenylalanine from the GM) decreased in the Epimedium group, indicating that Epimedium had effects on metabolism of phenylalanine and improved the status of GM.

## Conclusions and perspectives

GC is widely and successfully used in a variety of inflammatory conditions as an immunosuppressive agent, despite the long-term or high-dose use of GC is associated with detrimental effects on bone, leading to GIOP and increased fracture risk. With the increasing understanding of GM–host interactions in recent years, the involvement of GM has become an ingenious and non-negligible way to regulate the host health. The apparent association between the GM and bone metabolic processes suggests that the characterization and identification of GM features may have great clinical potential. Based on the proven regulatory effects of GC on GM, as well as GM on OP, the pivotal contribution of GM in maintaining the balance between the bone formation and bone absorption in the GIOP has become a research hotspot. Amount of clinical and animal researches indicated the beneficial effects of probiotics, prebiotics, traditional Chinese medicines, and bioactive substances derived from natural products on the treatment of GIOP. On this basis, we should also focus on whether GM plays a modulatory role in primary OP and GIOP via the same mechanisms, and how GM and its metabolites affect primary OP and GIOP through different ways and mechanisms in the future researches. Over and above, there are still challenges in translating the applications of GM from the animal studies to clinical practice, including the safety, efficacy, duration of use, and methods for making rational use in humans. Nonetheless, it is reasonable to realize that with further research development in the future, GM and its metabolites may become an important target for regulating the bone metabolism, which is expected to provide a novel approach for the prevention and clinical treatment of GIOP.

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## Declarations

**Conflict of interest** All the authors declare that they have no potential conflict of interests, nor biomedical financial interests.



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