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专论与综述

微生物-肠-骨轴与骨质疏松疾病的研究进展

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摘　要：肠道微生物被称为人体的“第二基因库”，对骨质疏松症的治疗具有良好的辅助作用。其主要通过肠上皮屏障、免疫系统、内分泌系统及肠道菌群代谢产物等途径在肠-骨轴的作用下影响肠道与骨代谢之间的联系。新型的肠道微生物靶向疗法如益生菌、益生元和膳食补充剂已被证明可有效预防骨质流失，但其长期疗效及安全性仍需进一步加强。因此，本文结合国内外研究进展，就微生物-肠-骨轴在骨质疏松症中的主要作用进行探讨，为骨质疏松症的治疗提供新的思路。
关键词：肠-骨轴；骨质疏松症；肠道微生物；菌群移植

Research progress in microbe-gut-bone axis and osteoporosis

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Abstract: Gut microbes, known as the body’s “second gene pool”, play a role in the treatment of osteoporosis. They affect the connection between intestinal tract and bone metabolism through intestinal epithelial barrier, immune system, endocrine system, and intestinal flora

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metabolites in the gut-bone axis. Novel gut microbiota-targeted therapies such as probiotics, prebiotics, and dietary supplements have been shown to be effective in preventing bone loss. However, their long-term efficacy and safety still need to be further enhanced. Therefore, this paper discusses the main role of the microbe-gut-bone axis in osteoporosis, which is expected to provide new ideas for the treatment of this disease.

Keywords: gut-bone axis; osteoporosis; gut microbiota; fecal microbiota transplantation

骨质疏松症作为全身代谢性骨病，以骨微结构破坏、骨量低及骨脆性增加而易发生骨折为特点，已成为备受现代社会广泛关注的公共性问题之一[1]。据流行病学调查结果显示，骨质疏松症已经成为老年人残疾和死亡的主要因素之一，我国年龄在 50 岁以上的居民的骨质疏松症发病率为 19.2%，65 岁以上的居民为 32.0%，并且超过 90.0%的患者对自己的病情毫不知情[2]。目前，现代医学对骨质疏松症的治疗虽有一定的临床疗效，但仍存在患者依存性差及胃肠道不良反应等问题。肠道微生物为定居于肠黏膜表面的大量共生性及致病性的微生物群，包括细菌、古生菌、病毒、真菌等。因其含有 10^{14} 种菌，所包含的基因量是人体的 150 倍之多，被称为“被遗忘的人体器官”[3]，肠道微生物主要由拟杆菌门(*Bacteroides*)和厚壁菌门(*Firmicutes*)构成[4]，虽然由于菌群间的相互作用致使肠道微生物长期处于动态平衡，但仍会因长时间的持续刺激或因肠道菌群中产生大的干扰因素而改变，已被证明对肠道微生物动态平衡有影响的因素包括衰老、饮食、环境、生理状态及口服抗生素的长期治疗[5]。肠道微生物与宿主之间的动态平衡一旦被破坏，骨质疏松症的患病率也会随之增加。研究表明，肠道微生物通过内分泌系统、免疫系统及其代谢产物等途径调节骨骼代谢[6]。如图 1 所示，本文在已有微生物-肠-骨轴研究基础上[7]，讨论并绘制了肠道微生物群与骨质疏松症之间的联系与潜在机制，以期为骨质疏松症的治疗提供新的思路。

1 微生物-肠-骨轴

胃肠道富含丰富的细菌与真菌群落，可以有效调节人体的新陈代谢和免疫状态。近年来，越来越多的学者提出“微生物-肠-骨轴”这一新的理念来解释肠道微生物与骨骼疾患之间的复杂关系[8]。肠道微生物广泛参与多种激素、代谢产物及细胞因子等多种活性产物的合成及释放，进而影响骨骼的吸收与破坏。随着年龄的增长，肠道微生物中致病性变形杆菌(*Proteus*)和拟杆菌数量增多，抗炎乳酸杆菌(*Lactobacillus*)数量减少，导致体内炎症反应，增加骨质疏松症的患病率[9]。目前，研究认为肠道微生物能够通过肠道上皮屏障、代谢产物、免疫系统及内分泌系统直接或间接地对“肠-骨轴”进行调控。

1.1 肠道屏障的完整性和钙吸收

肠道上皮屏障将肠道与有害抗原与病原体相分离，影响营养吸收并发挥免疫保护相关作用，由于肠道上皮细胞被紧密连接蛋白封闭，肠道微生物失调可通过破坏紧密连接蛋白结构的完整性并诱导肠上皮细胞凋亡，提高肠道屏障的通透性，使肠道微生物从肠腔转移到上皮下结构，诱导肠道炎症反应，导致 Th17 细胞增殖和破骨细胞相关因子 TNF-α、RANKL 和 IL-17 高表达，从而导致骨质流失[10]。Ma 等[11]研究表明，卵巢摘除(ovariectomized, OVX)大鼠肠道微生物结构及功能显著发生改变，而且 OVX 大鼠回肠上皮绒毛缩短及紧密连接蛋白表达下降，进而损害肠上皮屏障通透性并激活免疫通路，刺激

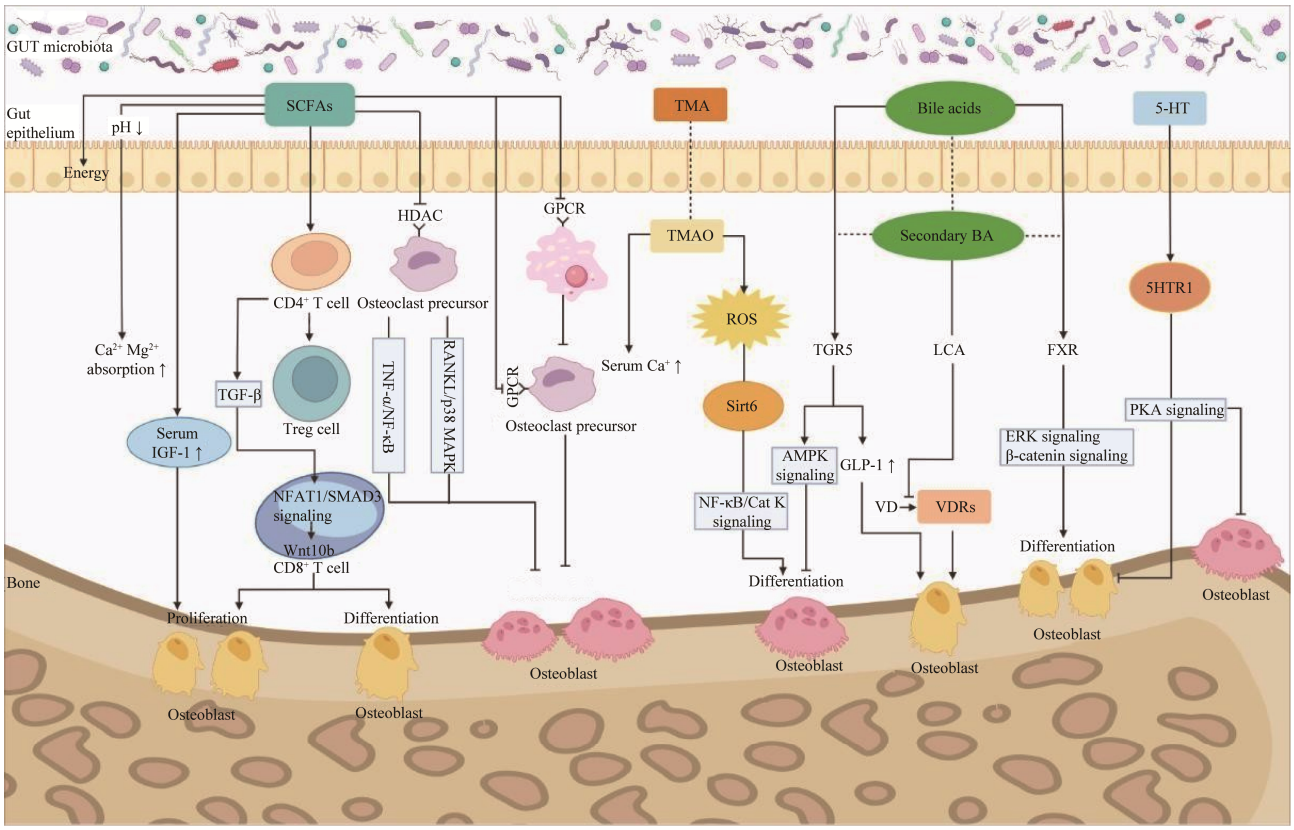

图 1　微生物-肠-骨轴机制图

Figure 1　Schematic diagram of the microbe-gut-bone axis.

$CD4^+$ T 细胞分化及破骨细胞形成。肠上皮屏障功能障碍同样会导致脂多糖(lipopolysaccharide, LPS)水平升高并且增强 *rank*、*traf6* 和 *cox-2* 信号传导及 Erk1/2 和 Jnk 磷酸化，促进破骨细胞分化[12]。

肠道微生物同样可影响骨骼发育所需的钙及维生素 D 等营养物质的吸收，肠道微生物能够将膳食纤维转化为短链脂肪酸(short-chain fatty acids, SCFAs)，并增加大鼠结肠中钙的转运，促进骨骼钙吸收[13]。乳酸杆菌和双歧杆菌属(*Bifidobacterium*)能够充分吸收与利用钙、磷、镁等微量元素，从而促进钙结合蛋白表达及骨矿物质化[14]。研究证明，肠道微生物可通过促进乳酸和 7-脱氢胆固醇的合成显著促进循环 25-羟基维生素 D 水平，以促进骨骼钙吸收[15]。此外，肠道微生物被认为是维生素 B 和维生素 K 的主要来源之一，在骨稳态中发挥着重要的作用[16]。

1.2　肠道菌群代谢产物

1.2.1　短链脂肪酸

肠道微生物能够将不被宿主消化酶分解代谢的膳食纤维通过氨基酸发酵产生 SCFAs，SCFAs 对骨的调节作用包括：(1) SCFAs 能够促进肠上皮屏障的形成[17]。(2) 溶解矿物质中的络合钙降低肠环境的 pH，使血清中游离钙水平增加并减少钙磷复合物的合成，促进骨骼对钙离子的吸收[18]。(3) SCFAs 通过激活 G 蛋白耦联受体 GPR41、GPR43 和 GPR109A，并能与其内源性配体游离脂肪酸受体 2 (FFAR2)、游离脂肪酸受体 3 (FFAR3)相结合，干扰破骨细胞能量代谢并直接减少破骨细胞形成；还可通过抑制 Th17 细胞在小肠的产生及 IL-6、IL-17 和 IL-23 炎性因

子表达，减少破骨细胞生成[19]。而且，SCFAs 可通过抑制组蛋白去乙酰化酶(histone deacetylase, HDAC)表达，并激活 TNF-α/NF-κB 和 RNAKL/p38 MAPK 通路抑制破骨细胞前体细胞对破骨细胞的分化[20]。据报道，SCFAs 通过增加 $CD4^+$ T 细胞和 Treg 细胞中 TGF-β1 水平促进成骨，进而促进 *nfat1*/*smad3* 转录复合物介导的 $CD8^+$ T 细胞中 Wnt10b 的分泌[21]。而且 SCFAs 可通过增加血清 IGF-1 水平，促进成骨细胞增殖和分化[22]。丁酸可以扩大肠道吸收面积，促进钙吸收，激活 *wnt* 信号通路和抑制 RANKL 诱导破骨细胞形成，丁酸能够通过介导甲状旁腺激素(parathyroid hoirmone, PTH)激活 *wnt* 信号促进骨形成[23]。Katono 等[24]研究证明，SCFAs 可以明显增加成骨细胞矿化结节的形成、矿化结节的含量、骨唾液蛋白、骨桥蛋白及骨保护素的表达。Tyagi 等[21]研究证明，丁酸通过激活 *wnt* 信号通路刺激骨形成，抑制破骨细胞分化。通过 SCFAs (丙酸和丁酸)灌胃治疗的小鼠能够通过诱导破骨细胞代谢重编程，导致破骨细胞活性下调，增加其骨量并能防止绝经后和炎症引起的骨丢失[25]。

1.2.2 氧化三甲胺

肠道代谢物氧化三甲胺(trimethylamine N-oxide, TMAO)是由三甲胺经含肝黄素的单加氧酶氧化而成。近年来，随着肠道微生物研究的深入，证明 TMAO 与心血管疾病和骨质疏松症密切相关[26]。TMAO 通过上调 NF-κB p65 表达提高活性氧(reactive oxygen species, ROS)、IL-1β、IL-6 和 TNF-α 水平，减缓骨髓间充质干细胞(bone mesenchymal stem cells, BMSCs)增殖，并且可以诱导 Pparγ 和 C/ebp-α 的表达上调促进 BMSCs 成脂分化，使成骨分化蛋白 Runx2 和 Opn 的表达下调[27]。而且，TMAO 可通过促使 ROS 积累激活 NF-κB/ctsk 信号通路，促进破骨细胞分化，致使骨量减少[28]。研究显示，TMAO 进入细胞后以 ADP 为激动剂激活 *ip3* 通路，促进细胞内 Ca^{2+}库中 Ca^{2+}的释放，使血清中 Ca^{2+}浓度增加，致使骨骼中 Ca^{2+}流失[29]。

1.2.3 5-羟色胺

5-羟色胺(5-hydroxytryptamine, 5-HT)又称为血清素。脑源性 5-HT 通过激活下丘脑腹内侧核神经元上的 5-HT2C 受体诱导骨形成；随后促进成骨细胞增殖，同时抑制破骨细胞的形成[30]；肠源性 5-HT 由肠嗜铬细胞和黏膜肥大细胞分泌产生，除了能够激活前成骨细胞上的 5-HT 受体 1B (HTR1B)外，还可与其相结合抑制环磷酸腺苷(cyclic AMP, cAMP)的产生，以及蛋白激酶 A (protein kinase A, PKA)介导的 cAMP 反应元件结合磷酸化，导致细胞周期蛋白(cylins, Cyc)基因的表达降低，进而抑制成骨细胞增殖[31]。Roshchina[32]研究证明，链球菌属(*Streptococcus*)、棒状杆菌属(*Corynebacterium*)和大肠杆菌(*Escherichia coli*)等细菌已被证实能产生 5-HT。Yano 等[33]研究证明，无菌(germfree, GF)小鼠 5-HT 水平降低，小梁骨体积/组织体积增加。Yadav 等[34]研究证明，肠道微生物能通过 *htr1b/pka/creb/cyclins* 信号调节肠道 5-HT，进而调节成骨细胞增殖和骨形成。

1.2.4 胆汁酸

胆汁酸通过“肠肝循环”在厌氧菌作用下由初级胆汁酸转变为次级胆汁酸，胆汁酸能够通过与法尼酯衍生物 X 受体(farnesoid X receptor, FXR)、G 蛋白偶联胆汁酸受体(G protein coupled bile acid receptor 5, TGR5)等相结合，并介导相关信号通路，从而在肠道微生物、糖脂代谢及免疫系统产生作用[35]。胆汁酸通过与 TGR5 结合可促进 cAMP 合成，激活蛋白激酶 A 途径的信号传导[36]，并且胆汁酸可直接刺激胰肠道内分泌细胞分泌 GLP-1[37]。现代医学研究证明，GLP-1 能够通过增加成骨细胞数量及上调骨形成相关

基因 *runx*2、*alp*、*coll* mRNA 和 *opg* mRNA 表达上调, 促进 BV/TV 及 Tb.N 增加, Tb.Sp 和 Tb.Pf 降低[38], 单羟基二次石胆酸(lithocholic acid, LCA)是肠道细菌脱羟基后产生的一种胆汁酸衍生物, 并且作为维生素 D 受体(VDR)能够增加 *opg* 和 *rankl* 的基因表达, LCA 过度沉积可破坏成骨细胞线粒体的活性, 影响骨形成[39]。此外, LCA 能够明显降低成骨细胞中维生素 D 的作用, 而且能与骨钙素、骨保护素及 *rankl* 基因表达降低相关[40]。

2 肠骨轴调节免疫改善骨质疏松症

肠道微生物能够通过改善宿主免疫状态调节骨代谢。Hao 等[41]研究发现, 肠道微生物紊乱可促使 $CD4^+$ T 细胞亚型 Th17 细胞的产生与分化, 分泌 IL-1、IL-17a、IL-6、低水平的干扰素-γ (interferon-γ, IFN-γ) 和肿瘤坏死因子(tumor necrosis factor, TNF), 促使 RANKL 释放, 形成破骨细胞, 加剧骨质流失。调节性 T 细胞(regulatory T cell, Treg)刺激骨髓 $CD8^+$ T 细胞产生成骨的 *wnt* 信号通路配体 Wnt10b 蛋白, 从而促进成骨分化[42]。而且能通过抑制 Traf6 和 Nfatc1 的表达, 以及 RANKL 诱导的 NF-κB 信号通路的激活, 减少破骨细胞生成, 促进骨形成和再生[43]。肠道微生物通过调节 Th-17/Treg 之间的动态平衡, 增加 TGF-β 和 IL-10 等抗炎因子的分泌, 抑制破骨细胞增殖和分化, 减少骨吸收, 增加骨量, 防治骨质疏松症[44]。脆弱拟杆菌(*Bacteroides fragilis*)可促进肠道 $CD4^+$ T 细胞向 $Foxp3^+$Treg 细胞转化, 促进肠黏膜免疫耐受[45]。Atarashi 等[46]研究发现, 梭状芽孢杆菌属(*Clostridium prazmowski*)菌株定殖, 能够促进 Treg 细胞增殖和 TGF-β 表达, 抑制破骨细胞形成。$CD4^+FOXP3^+$Treg 细胞与全身性免疫相关, 梭状芽孢杆菌属定殖于诺维氏梭菌病小鼠体内导致结肠 Tregs 的积累[47]。此外, $CD4^+CD25^+$ $Foxp3^+$Treg 细胞能够通过细胞毒性 T 淋巴细胞相关蛋白 4 (cytotoxic T-lymphocyte-associated protein 4, CTLA-4)介导的途径抑制破骨细胞成熟/分化。Dar 等[48]研究证明, 克劳氏芽孢杆菌(*Bacillus clausii*)增加 OVX 小鼠中的 Treg 细胞并促进骨形成。Jia 等[49]研究证明, 益生菌的胃内给药分别通过降低 $CD4^+IL\text{-}17A^+$Th17 细胞比率, 增加来自骨髓的 $CD4^+CD25^+Foxp3^+$Treg 细胞比率, 恢复 Th17/Treg 细胞平衡。Rubinstein 等[50]研究证明, 梭杆菌属(*Fusobacterium*)和脆弱拟杆菌可以激活 *wnt*/*β-catenin* 信号转导, 促进骨形成。鼠李糖乳杆菌(*Lactobacillus rhamnosus*)治疗通过 Treg 细胞介导的 $CD8^+$T 细胞 Wnt10b 产生的调节来调节骨合成代谢[21]。益生菌能够抑制 NF-κB、IκB 激酶-α、TLR2、TLR6、IL-8 以及 TNF-α 的表达[51], 肠道丝状菌(segmented filamentous bacteria, SFB)能够增加 IFN-γ 和 IL-17 的产生, 其在骨形成与骨重塑中有重要作用, 能够有效防治 OVX 小鼠的骨质疏松症症状[52]。

3 肠骨轴调节内分泌改善骨质疏松症

肠道微生物就像人体的一种“虚拟内分泌器官”, 可以通过与内分泌系统相互作业调节骨代谢[53]。

Li 等[54]研究证明, 雌激素缺乏导致肠骨常规饲养 C57BL/6J 小鼠肠道微生物多样性下降, Th17 细胞分化度增高, 肠道屏障通透性下降, 同时上调小肠及骨髓中的破骨细胞分化因子 TNF-α、RANKL、IL-17 表达, 导致骨小梁减少, 骨密度下降; 而无菌小鼠雌激素缺乏却无法导致骨丢失, 并且通过给常规饲养 OVX 小鼠行鼠李糖乳杆菌灌胃治疗能明显改善上述症状、预防骨

质疏松症。

甲状旁腺激素能有效促进骨形成和骨吸收，主要取决自靶细胞间歇性还是连续性暴露于PTH。Kim 等[55]研究证明，给予常规饲养C57BL/6J 小鼠注射间歇性甲状旁腺激素(iPTH)能明显增加其骨小梁体积分数及骨小梁厚度，但无菌小鼠无明显改变。肠道微生物能增加 iPTH所需大丁酸盐浓度，并且提高骨髓中 Tregs 水平，以防止骨质疏松症[56]。然而，在富含 *SFB* 的小鼠中能触发持续性甲状旁腺激素(cPTH)，通过调节 *opg*/*rankl*/*rank* 通路和诱导单核细胞趋化蛋白-1 (MCP-1)的表达，从而触发破骨细胞生成性 Th17 细胞的募集，从而导致骨丢失，但在无菌小鼠中并无上述症状[56]。

胰岛素生长因子-1 (IGF-1)能通过内分泌、自分泌及旁分泌方式促进成骨细胞分化。Yan 等[57]研究显示，长期定殖肠道微生物的无菌小鼠能通过增加 IGF-1 的产生促进骨形成，而常规饲养小鼠通过抗生素治疗，其血清 IGF-1 及骨小梁水平有所降低。Ohlsson 等[58]研究证明，通过给予OVX 小鼠乳酸杆菌，其骨吸收标志物水平明显下降，骨形成明显改善。高伟华[59]研究证明，通过乳酸杆菌灌胃治疗的糖尿病小鼠能明显提高其益生菌丰度，缓解胰岛素抵抗状态，从而延缓骨质疏松症进程。Amar 等[60]研究证明，通过双歧杆菌和乳酸杆菌灌胃治疗糖尿病小鼠后，能够明显改善其肠道微生物，降低 TNF-α、IL-1β、PAI-1、IL-6 和 IFN-γ 的蛋白表达并改善胰岛素敏感性，进而促进骨形成与修复。Schwarzer 等[61]研究证明，通过给予营养不良小鼠行植物乳杆菌(*Lactobacillus plantarum*)灌胃治疗，能通过增加IGF-1 活性促进小鼠骨骼生长。Avella 等[62]研究证明，通过给予斑马鱼行鼠李糖乳杆菌灌胃治疗，能明显提高 *igf-1* mRNA 的表达及斑马鱼骨干钙化。

4 粪菌移植和益生菌/益生元疗法

肠道菌群为靶向目标的细胞生物疗法现已成为代谢性疾病治疗方案的主要研究方向。针对这一治疗靶点，粪菌移植和益生菌或益生元的辅助治疗已成为现在的热点研究，粪菌移植是通过将粪便从健康供体移植到接受体的肠道内，从而达到重建肠道微生物群，阻滞临床疾病进程。目前，粪菌移植(fecal microbiota transplantation, FMT)已在炎症性肠病、肠易激综合征、便秘、肝病、孤独症和癫痫等疾病的治疗中效果显著[63]，Ma 等[64]通过对 18 个月的雌性 SD 大鼠进行粪菌移植治疗，结果发现，老年性骨质疏松大鼠肠道微生物群与供体大鼠相似且骨质疏松症状明显得以改善。除了粪菌移植，由于骨质疏松症常会合并胃肠道相关疾患，而益生元或益生菌能够重塑肠道微生物群并通过调节免疫系统、肠道屏障及其代谢产物等作用，因此，大量的试验研究也集中于益生菌对骨质疏松症的治疗机制。目前，临床试验已能够证实益生菌或益生元能够通过重塑肠道微生物群，显著改善骨质疏松症患者的临床症状且具备良好的安全性[65]。

5 总结与展望

随着社会人口老龄化的加剧，骨质疏松症已成为我国甚至全球需要面对的重要卫生问题。肠-骨轴作为骨代谢与胃肠道之间的“纽带”，能够通过肠道微生物释放雌激素的类似物、血清素等小分子物质、调节钙磷的代谢进而改善骨代谢。随着年龄的增长，老年人所摄入的膳食纤维减少，慢性疾病的患病率增长，营养摄入不均衡，导致老年人肠道微生物多样性降低，扰乱肠道代谢和免疫机制的平衡，从而影响骨代谢，致使骨质疏松症形成。虽然已有粪菌移植及益生菌等治

疗方法的提出，但粪菌移植的无菌操作要求较高，益生菌菌群的特异性、剂量及干预时间等较难衡量。因此，未来可采用多学科交融深入研究肠道微生物与骨代谢之间的联系，同时开展益生菌治疗或辅助治疗肠道微生物患者的临床研究，筛选出能改善骨代谢的优良菌株，为预防、延缓及改善肠道微生物提供临床指导。

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