Bone & Joint Research

Supplementary Material

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Table i. Summary of the study characteristics.

Source	Year	Animal	Induction of osteoporosis	Determination of gut microbiota	Intervention	Assessment	Findings
Sjögren et al.[1]	2012	C57BL6/J Mice	None	None	None	1. Peripheral quantitative computed tomography (pQCT) and micro-CT: trabecular BMD cortical bone parameters; 2. Histomorphometry：static parameters and dynamic parameters 3. ELISA: testosterone; calcium; 25-Hydroxy Vitamin D; PTH;	1. Germ-free (GF) mice exhibit increased bone mass associated with reduced number of osteoclasts per bone surface, reduced expression of inflammatory cytokines in bone and bone marrow compared with conventionally raised (CONV-R) mice. 2. Colonization of GF mice with a normal gut microbiota normalizes bone mass, and the decreased frequency of $CD4^+$T cells and $CD11b^+$/GR 1 osteoclast precursor cells in bone marrow.

						serotonin, type 1 collagen fragments-Serum level of osteocalcin 4. qRT-PCR: (Colon)Tph-1, T1R, L-32F, L-32R; (femur): IL-6, IL-1,TNF-α 5. Flow cytometry: CD4(T helper cells); CD8(cytotoxic T cells), CD11b(OCL precursor) 6. In vitro culture of osteoclasts from bone marrow cells: TRAP staining	
Cho et al.[2]	2012	C57BL/6J Mice	None	Real-time quantitative PCR for taxonomic and functional analyses	Early life (age of 4 weeks) subtherapeutic antibiotic treatment	1. dual-energy X-ray absorptiometry (DEXA)：BMD and body composition 2. serum hormone: GIP, insulin, IGF-1, peptide YY, ghrelin, and leptin by Magnetic Bead Panel analyzer. 3. Glucose tolerance test: 4. Hepatic triglyceride measurements 5. qPCR: Gene set enrichment analysis of C2 and C5 pathway 6. Histological and immunohistochemical analyses of the fat tissue	Administration of subtherapeutic antibiotic therapy in early life alters intestinal microbiota (elevated ratio of Firmicutes to Bacteria) and SCFA metabolism, increase adiposity and BMD.
Ohlsson et al.[3]	2014	C57BL/6N mice	OVX	None	Lactobacillus (L) strain, L. paracasei DSM 13434 (L. para) or a mixture of three strains-L. paracasei DSM 13434, L. plantarum, L. plantarum DSM 15312 and DSM 15313 (L. mix) given in the drinking water	1. pQCT(Cortical bone parameters): Tt ar, Crt Thk 2. micro-CT: Trabecular bone parameters 3. real-time PCR (Cortical bone and bone marrow): IL-6, IL-1β, TNF-α, RANKL, OPG, Osterix, Collα1, osteocalcin, TGFβ1. 4. ELISA and other assays (Serum and Urine): calcium, creatinine, 25-Hydroxy Vitamin D, C-terminal telopeptides, osteocalcin (immunoradiometric assay)	1. Treatment with L. para or the L.mix prevents OVX-induced cortical bone loss. 2. These probiotic treatments alter the immune status in the bone resulting in attenuated bone resorption in OVX mice.

						5. Flow Cytometry: CD4, Foxp3	
Britton et al.[4]	2014	Balb/c mice	OVX	16S sequencing	Gavage with L. reuteri ATCC PTA 6475 or MRS broth	1. μCT bone imaging：trabecular and cortical parameters of femur 2. dynamic histomorphometric measures 3. PCR:(femur) Trap5b and HPRT, RANKL, Osterix 4. Serum TRAP5b and Osteocalcin measurement 5. Flow cytometry: immune cell populations in bone marrow.	L. reuteri treatment significantly protected OVX mice from bone loss. Osteoclast bone resorption markers and activators (Trap5 and RANKL) and osteoclastogenesis are significantly decreased.
Li et al.[5]	2016	Conv. R C57BL6/J mice; Germ-free C57BL6/J mice	None	None	1. Colonization of GF mice. 2. sex steroid–depletion by Leuprolide administration Lactobacillus rhamnosus GG (LGG) or VSL#3, non-probiotic strain of E. coli or a mutant LGG supplementation	1. Intestinal permeability assays 2. micro CT: trabecular and cortical parameters 3. Quantitative bone histomorphometry: 4. ELISA: IL-17, TNF, RANKL, IFNy, and IL-4; serum CTX and serum osteocalcin 5. Flow cytometry: (BM, spleen, and SILP) TCRβ, CD4, CD8, IL-17, TNF, RANKL qPCR (BM, BM T cells, and small intestine): expression levels of murine Il17, TNF, and RANKL	1. In germ-free (GF) mice, sex steroid deficiency failed to increase osteoclastogenic cytokine production, stimulate bone resorption, and cause trabecular bone loss. 2. Supplementation of the normal flora of sex steroid–depleted mice with the commonly used probiotics Lactobacillus rhamnosus GG (LGG) or VSL#3 significantly tightened intestinal barrier integrity and completely protected mice against sex steroid depletion–induced bone loss

Yan et al.[6]	2016	GF F1 hybrid CB6F1 mice; BALB/c mice	None	Real-time PCR	1. Antibiotics treatment for 1 month: either mixture of antibiotics (ampicillin, vancomycin, metronidazole, and neomycin), or vancomycin 2. SCFA Supplementation antibiotics mixture for 2 W to deplete microbiota, and then either SCFA or sodium control (150mM sodium chloride) was added to the antibiotics water for another 4 W 3. Colonization of GF Mice with feces from CB6F1 SPF mice	1. Micro CT: BMD and 3D microstructural properties of the Femur 2. Histomorphometry: Dynamic bone formation parameters including MAR, BFR 3. Histology: Growth plate thickness measurement and toluidine blue staining Growth plate thickness 4. Real-time PCR (liver, fat pad, gastrocnemius muscle, bone, and bone marrow): Igf1, Igfbp3, Igf1r, Runx2, etc. 5. ELISA: Tissue IGF-1, Serum IGF-1 IGFBP3, GH, CTX-1, and P1NP 6. SCFA measurement of the cecal content 7. Gas chromatographic analysis 8. Metabolic cage measurements 9. Bomb calorimetry	1. Short-term colonization of adult GF mice promotes both bone formation and resorption by osteoclastogenic cytokines, long term colonization leads to increased bone growth. 2. Cecal SCFA (a major metabolite produced by gut microbiota during fermentation of dietary fiber) concentration is altered by colonization and antibiotic treatment 3. SCFA increases systemic IGF-1 levels and restores bone mass in antibiotic-treated mice.

Guss et al.[7]	2017	1. Toll-like receptor 5-deficient mouse TLR5KO (deficits in the immune system) 2. Wild-type mouse C57BL/6J	None	16S rRNA gene sequencing qPCR	Treatment group received broad-spectrum antibiotics (1.0h/L ampicillin and 0.5g/L neomycin)	1. Micro-CT: Cortical bone (Femoral diaphyseal): total area, cortical cross-sectional area, cortical thickness, marrow area, and moment of inertia; Trabecular bone (Tibia): BV/TV, Tb.Th, Tb.Sp, and (Ct.TMD) 2. Mechanical test: loaded to failure; force and displacement; bending stiffness 3. Colon Histology: lymphoid aggregate size, lymphoid aggregate density, apoptotic cells per high powered field, and presence of inflammation. 4. Flow cytometry: relative percentages of B and T cells in spleens	1. B and T cell populations were depleted in TLR5KO mice and ΔMicrobiota mice. 2. Alterations in gut microbiota for extended periods during growth may lead to impaired whole bone mechanical properties.
Ohlsson et al.[8]	2017	8 groups Conventional C57Bl6/J mice (CONV-R) or Germ-free mice (GF mice) - WT mice - MyD88 mice - Nod 1 mice - Nod 2 mice	None	None	None	1. High-resolution micro-CT: cortical measurement Ct. th. 2. PT-PCR(Bone): TNFα, RANKL, CTSK	The expression of TNFα and the osteoclastogenic factor RANKL in bone was reduced in GF compared to CONV-R WT mice but not in $Nod1^{-/-}$ or $Nod2^{-/-}$, which indicated that GM regulates bone loss through NOD1 and NOD 2 signaling.

Villa et al.[9]	2018	C57BL/6J female	None	Quantitative PCR analysis：Counts of total bacteria, Bacteroides and Prevotella	AIN93G diet with high or low vitamin D from before mating until weaning. The male and female offspring continue the vitamin D level or switch	1. Dual-energy X-ray absorptiometry: 2. Micro-CT: distal femur, femur midpoint, lumbar vertebrate 3. Mechanical test: peak load of bone strength 4. LC-MS: serum vitamin D 5. Immunohistochemistry of VDR expression	Dietary vitamin D programs Bacteroides in male adult offspring only, which correlated negatively with systemic inflammation and positively with bone strength and structure.
Quach et al.[10]	2018	GF Swiss Webster mice and GF C57BL/6 mice	None	16S rRNA gene amplification, and sequencing.	Cecal and fecal samples from conventionally raised Swiss Webster (CONV-D) and C57BL/6 mice, or human was inoculated to the GF animals	1. MicroCT（Femur; trabecular bone: Bone volume fraction (BVF), tissue mineral density, Tb. Th), trabecular number (Tb.N.), and trabecular spacing (Tb. Sp), Cortical bone parameters 2. Static and dynamic histomorphometry: BFR and mineral apposition rate (MAR) measurements 3. qRT-PCR (colon): HPRT, TNF-α, IL-1, IL-6 4. Flow cytometry (Bone marrow cells): CD3，CD4，CD8a，GR1/Lys-6G，CD11b	1. In spite of the successful colonization of GF mice with the gut microbiota of either mouse or human origin, bone mass did not change significantly in any of the groups tested. 2. Static and dynamic bone parameters and osteoclast precursor and T cell populations, the expression of several inflammatory markers, were mostly unchanged following microbial colonization of GF mice.

Tyagi et al.[11]	2018	C57BL/6 WT； TCRβ−/−； C57BL/6-Tg(TcraTcrb)1100Mjb/J； C57BL/6-Tg(Foxp3-DTR/EGFP)23.2Spar/Mmjax	None	16S rRNA gene amplification, and sequencing	Lactobacillus rhamnosus GG or butyrate supplementation Anti-CD25 Ab treatment Diphtheria Toxin (DT) treatment	1. μCT measurements: femur trabecular and cortical bone 2. Quantitative bone histomorphometry 3. LC-MS/MS: Butyrate measurements 4. ELISAs—P1NP, osteocalcin, and CTX 5. Real-time RT-PCR (bone marrow): Wnt10b etc. 6. Flow cytometry and cell sorting: CD16/32 etc. 7. Western blot: BM CD8+ T cells	Butyrate produced in the gut following LGG ingestion or butyrate fed directly regulates bone anabolism via Treg cell-mediated regulation of CD8+ T cell Wnt10b production.
Schepper et al.[12]	2019	BALB/c mice	None	16S rRNA gene amplification, and sequencing	1. Broad-spectrum antibiotics: ampicillin and neomycin for 2 weeks and 2. L. reuteri 6475 (LR), Lactobacillus rhamnosus (LGG), nonpathogenic Escherichia coli, or 1.25% MDY (mucus supplement)	1. Micro CT bone imaging 2. real-time PCR(Tibia): *HPRT,* Osteocalcin, TNF-a; IL-10 3. Histomorphometric measures: MAR, BFR, TRAP staining 4. ELISA: serum TRAP5b and osteocalcin 5. Mechanical testing 6. Ex vivo Ussing chamber intestinal permeability	Probiotic Lactobacillus reuteri prevents postantibiotic bone Loss by reducing intestinal dysbiosis and preventing barrier disruption.

Schepper et al.[13]	2019	C57BL/6J male mice(15-week)	Prednisolone treatment	16S rRNA gene amplification, and sequencing	L. reuteri 6475 LGG MDY treatment ABX + prednisolone Fecal transplantation from GC-treated mice	1. Micro CT 2. Histomorphometric measures: MAR, BFR, TRAP staining 3. ELISA: bacterial endotoxin 4. Mechanical testing 5. real-time PCR(Tibia): *HPRT, Bax, Bcl-2, Wnt10b* 6. Flow cytometry: $CD4^+$ in bone marrow	1. Long-term ABX prevented GC-induced trabecular bone loss, showing the requirement of gut microbiota for GIO. 2. Furthermore, transplantation of GC-treated mouse fecal material into untreated WT mice caused bone loss. 3. GC caused intestinal barrier breaks. as evidenced by increased serum endotoxin level, that was prevented by LR and ABX treatments
Liu. et al.[14]	2019	C57BL6/J	tenofovir disoproxil fumarate (TDF)	16S recombinant deoxyribonucleic acid pyrosequencing	Lactobacillus rhamnosus GG (LGG)+TDF, TDF, and zoledronic acid+TDF	1. Micro-CT 2. Dynamic histomorphometric analyses 3. Histology (femur and gut): H&E and TRAP. 4. Mechanical test 5. ELISA: Serum P1NP, RANKL, CTX-1, TNF-α, IL-17 6. Flow cytometry: spleen, BM, and mesenteric lymph node. 7. Intestinal permeability	LGG reconstructed the structure of the GM, promoted the expression of lysophosphatidylcholines, and improved intestinal integrity to suppress the TDF-induced inflammatory response, which resulted in attenuation of TDF-induced bone loss in mice.
Tousen et al.[15]	2019	Female mice (ddY strain)	OVX	16S rRNA sequencing	High amylose corn starch AH-HAS supplemented in diets	1. BMD of the femur was quantified by dual-energy X-ray absorptiometry 2. Microcomputed Tomography (μCT) Analysis of the Distal Femur 3. ELISA: C-terminal telopeptide of type I collagen (CTX-I) in urine 4. Real-Time PCR (Intestine Tissue or the Bone Marrow of the Tibia): IL-10, NF-***κ***B, IL-7, etc.	AH-HAS might change the microbiota (increased abundance of Bifidobacterium spp) and immune status (upregulate IL-10 and downregulate NF-***κ***B, IL-7) of the bone marrow, resulting in attenuated bone resorption in OVX mice.

Zhao et al.[16]	2019	SAMP6 mice; SAMR1 mice	None	16S rRNA sequencing	Eclipta prostrata solution for 12 weeks Oral Lactobacillus bulgaricus CICC6045 Lactococcus lactis NZ9000 supplementation	1. micro-CT 2. western blot detection the bone-relating factor (Bone marrow): RunX-2, OPG, RAnKL, PPARγ, TRAP5b and ALP	1. Bone microstructure was significantly improved by E. prostrata. 2. Sequencing results indicated that E. prostrata altered the bacterial community. 3. The abundance of bacteria genera Lactobacillus and Lactococcus was markedly decreased in individuals with OP and positively correlated with high dose of E. prostrata.
Tavakoli et al.[17]	2019	Townes SCD mice	None	16S rRNA sequence	A four-drug cocktail of antibiotics (ampicillin, vancomycin, neomycin, and metronidazole)	1. Dual-beam X-ray absorptiometry (DEXA) BMD 2. μCt scanning of femurs 3. Bone histomorphometry: Osteoclast number/bone surface, interlabel thickness, MAR, MS/BS, and BFR/BS. 4. ELISA: serum calcium, phosphate, 1,25-Dihydroxy Vitamin D; Osteocalcin Creatinine; Serum inflammatory cytokines IL10, INFγ, and IL27. 5. Rt-qPCR (femur and small intestine): gene expression b-Actin, Eubacteria; Alp, Col1a1; Osterix; Runx2; Ocn; Rankl; Opg; Ctsk; Igf1; Tnfa; Il17; Ifng; Claudin3; Claudin15	Increased bacteria load augments antigenic load traversing the impaired intestinal barrier through inflammation, leading to increased inflammatory cytokines, impaired osteoblast function, and bone loss in SCD mice.

Tanabe et al.[18]	2019	SAMP6 mice	None	culture-dependent analytical methods	5% fructooligosaccharide (FOS), 5% glucomannan, or a control diet for 31 weeks.	1. Assessment of Femoral Bone using Soft X-rays: areas of high bone density of the femoral bone 2. Atomic absorption spectrophotometry: Calcium Concentrations in Experimental Diets, Feces, and Femoral Bones 3. ELISA: Serum Osteocalcin levels; CRP, urine deoxypyridinoline 4. Cytometric bead array method: serum TNF-α	Dietary intake of prebiotics including FOS and GM modified gut microbiota and reduced bone resorption by reducing systemic inflammation in SAMP6.
Guss et al.[19]	2019	C57BL/6 J mice	None	Metagenomic analysis	Broad-spectrum antibiotics for 12 weeks	1. Raman spectroscopy：Bone tissue crystallinity and mineral to matrix ratio，Reduced modulus，Hardness 2. ELISA: Osteocalcin protein in bone matrix 3. Liquid chromatography/ mass spectroscopy (LC/MS): phyllo-quinone (PK) and menaquinone (MK-4-13) concentrations in the cecum, liver and kidney	Shifts in functional capacity of the gut microbiota were associated with changes in bone mineral crystallinity, the degree of carbonate substitution, and concentrations of microbially-derived forms of vitamin K in the body.
Li et al.[20]	2019	Male ICR mice	Intraperitoneally injected with D-gal NaNO2 to induce premature aging.	16S ribosomal DNA sequencing	Vitamin E and Fructus Ligustri Lucidi (FLL) supplementation	1. μCT: TV, BV, BV/TV, Tb. N, Tb.Th, Tb.Sp, Tt.Ar, Ct. Ar, Ct. Th. 2. FTIR: Bone material properties 3. Bone biomechanical strength assay 4. Histology: H&E and safranin O/fast staining of femur	1. Aging phenotype is likely triggered by abnormal changes in the gut microbiota population of Bifidobacterium and the ratio of Firmicutes/ Bacteroidetes that resulted in increased TAMO. 2. FLL rescue osteoporotic bone phenotype and improve the cognitive function in aging mice, which may be linked to the regulation of gut microbiota diversity, antioxidant activity, and the levels of TMAO and Sirt6.

						5. IHC staining: Sirt6, CatK, acetyl-NF-***κ***B -p65 6. Western blot analysis: Nox4, FMO3, Sirt6 (NF-***κ***B -p65, and CatK. 7. LC/MS/MS analysis: Circulatory TMAO levels 8. Serum biomarkers determination: 8-OH-dG, MDA, TAC, GSH, GSSG, TNF-α, P1NP and CTx-1 9. Morris water maze test	
Hathaway-Schrader et al.[21]	2019	Sex-matched C57BL6/J SPF mice	None	Real-time PCR for the percentage and composition of specific phylum	Antibiotics treatment: vancomycin, imipenem/cilastatin, and neomycin from age of 6 weeks to 12 weeks	1. μCT: BMD and structural parameters 2. Static and dynamic histomorphometric analysis; TRAP staining 3. Serum biochemical assays: Osteocalcin, TNF-a, CCL3, CCL4 4. Quantitative Real-Time PCR: cytokine and antigen presentation gene expression in femur 5. Flow cytometry: (Bone marrow) CD4、CD3、CD8 etc. 6. Histopathology for kidney and livers	1. Antibiotic disruption of gut microbiota composition alters host immune response effects, which critically modulates normal osteoimmune processes in the postpubertal developing skeleton. 2. Antibiotic significantly increases in α-proteobacteria and γ-proteobacteria and a decrease in Bacteroidetes in male but increase in α-proteobacteria and decreases in Bacteroidetes and Firmicutes in female.
Rios et al.[22]	2020	Wild-type C57BL6/J mice; Lymphocyte deficient Rag2 (KO) C57BL6/J mice	Dysbiosis-induced bone loss	16S rRNA gene amplification, and sequencing	Ampicillin/neomycin cocktail in water for 2 weeks oral Lactobacillus reuteri treatment for 4 weeks (post-ABX treatment)	1. Micro-computed tomography (μCT) bone imaging 2. ELISA: Serum osteocalcin (OC) TRAP5b： 3. Mechanical testing	1. Mice treated with L. reuteri did not display bone loss, suggesting a bone protective role for this group of bacteria. 2. Lymphocytes played an important role in regulating post-antibiotic dysbiosis-induced bone loss.

Li et al.[23]	2020	CR mice	glucocorticoid-induced osteoporosis	16S rRNA gene sequencing	Tuna Bone Powder for 10 weeks	1. BMD and Bone Microarchitecture Measurement by micro-CT 2. Serum Biochemical Index: ALP, TRACP 3. ICP–MS: calcium content in fecal and femur 4. GC–MS: SCFAs in fecal samples 5. RT-PCR: gene expression of the NF-κB pathway and Wnt/β-catenin pathway in femur, colon and spleen tissue	TBP ameliorates GIOP in mice through: 1. regulating signaling pathways (inhibit NF-κB pathway and upregulate Wnt/β-catenin pathway); 2. blocking pro-inflammatory cytokines, 3. repairing the intestinal epithelial barrier, 4. modulating gut microbiota (SCFA producing bacteria increases).
Hathaway-Schrader et al.[24]	2020	C57BL/6 T strain 1. Germ-free 2. Specific pathogen-free 3. excluded-flora mice (SPF+SFB free)	None	16S rRNA gene sequencing	Segmented filamentous bacteria (SFB) colonization	1. micro-CT: Trabecular and cortical bone morphology 2. Histomorphometry: TRAP staining 3. Quantitative real-time PCR: osteogenic, immune, and cytokine gene expression in bone 4. Flow cytometric analysis: (Femur bone marrow, lymph node) 5. Serum biochemical assays: CTX-1, OCN, C3, IL17A, LCN2	SFB-colonized mice had an osteopenic trabecular bone phenotype, which appears to be mediated through immunomodulatory effects in both the gut and liver.
Parvaneh et al.[25]	2015	Sprague–Dawley rats	OVX	Enumeration of by CFU counting	1 mL of B. longum (10^8-10^9) (CFU/mL) via oral gavage daily	1. micro-CT analysis: BMD and microstructural parameters 2. Mechanical test 3. Bone Histology Assessment: HE staining and Static parameters: ObS/BS, OcS/BS, ES/BS, OS/BS, OV/BV 4. ELISA: Serum OC, CTX 5. Atomic absorption spectrophotometry: Calcium, magnesium, and zinc content of the femur 6. Real-Time PCR (fumer): Bmp-2, Sparc	B. longum alleviated bone loss in OVX rats and enhanced BMD by decreasing bone resorption and increasing bone formation by increasing ($p < 0.05$) the expression of Sparc and Bmp-2 genes.

Wang et al.[26]	2016	Wistar rats	OVX	16S rRNA high throughput sequencing	Ca-SGP (Sialoglycoprotein) supplementation	Dual-energy X-ray absorptiometry: BMD	Ca-SGP treatment significantly prevent bone loss and reversed the increase of Escherichia coli and Bacteroides fragilis, and the decrease of Clostridium leptum, Faecalibacterium prausnitzii, and Lactobacillus induced by ovariectomy.
Conley et al.[27]	2017	Sprague Dawley rats	OVX	16S amplicon sequencing	Low/high-dose nitrate supplementation	1. DEXA: BMD 2. Microcomputed tomography：Tibia metaphysis， epiphysis and midshaft 3. Histomorphometry: Fluorochrome-based measurements of bone formation 4. ELISA: Serum osteocalcin, C-terminal telopeptide 5. Rat Osteoporosis RT² Profiler PCR Array 6. Standard gas-phase chemiluminescence method Nitrate and nitrite content	1. OVX resulted in cancellous bone loss, increased bone turnover, and fecal microbiome changes. Increased Firmicutes and decreased Bacteroideceae and Alcaligenaceae. 2. Three weeks of nitrate supplementation does not slow bone loss or alter the fecal microbiome in OVX.

Eaimworawuthikul et al.[28]	2019	Wistar rats	high-fat diet (HFD)	None	HFD for 24 W, at 12W, rats were given either a vehicle, Lactobacillus paracasei HII01, (XOS), or symbiotic (L. paracasei HII01 and XOS) for an additional 12 W	1. Static histomorphometric parameters Goldner's trichrome staining: BV/TV, Tb.Th, Tb. N, Tb.Sp, (Ob.S/BS, %), (Oc.S/BS, %), aES/BS, % 2. Dynamic histomorphometric: MAR, BFR 3. Colorimetric assay：plasma glucose, triglyceride, and cholesterol levels 4. ELISA: plasma insulin 5. LAL (Limulus amebocyte lysate) testing：serum lipopolysaccharide 6. OGTT test	Probiotic *L. paracasei* HII01, prebiotic XOS, and the synbiotics equally improved metabolic disturbance, reduced systemic inflammation, increased trabecular thickness, decreased osteoclasts and active erosion surfaces and restored mineral apposition and BFR.
Guo et al.[29]	2019	Sprague Dawley rats	OVX	16S rRNA sequencing	Duck Egg White–Derived Peptide VSEE supplementation by gavage	1. Micro-CT: femur 2. Mechanical test (femur and tibia). 3. Histologic Analyses (Ileum, Liver, and Adipose Tissue): oil red O staining 4. ELISA: OCN, β-CTX, and PINP 5. Other Serum parameters analysis: calcium, TC, TG, HDL-C, LDL-C con liver TC, TG, LDL-C and HDL-C, and ALT activities	1. VSEE reverses bone loss and regulates dyslipidemia through Wnt/$\boldsymbol{\beta}$-catenin signaling pathway in OVX rats. 2. Firmicutes phylum, Veillonellaceae, Prevotellaceae and six genera in VSEE group are significantly different compared with the control group.

Xie et al.[30]	2020	Sprague Dawley rats	OVX	16S rDNA Gene Sequencing	Y1R antagonist BIBO3304 （ Intraperitoneal injection）	1. Micro-CT：BMD, Microarchitecture parameters 2. Histologic Assay: (femur)HE staining ELISA: Serum Estradiol, Calcium, and Phosphorus Measurement	1. The results indicated that OVX+ Y1R antagonist group showed significantly higher BMD, BV/TV, Tb. Th, Tb. N, Conn.D, and serum Ca2+ level than those in OVX group. 2. Y1R antagonist changed the gut microbiota composition with lower Firmicutes/Bacteroidetes ratio and higher proportions of some probiotics, including Lactobacillus.

List of acronyms:

8-OH-dG	8-Hydroxydeoxyguanosine
ALP	Alkaline Phosphatase
ABX	Broad-spectrum antibiotics
AH-HAS	Acid-Hydrolyzed-High Amylose Corn Starch
BAX	Bcl-2-Associated X
BCL	B-cell Lymphoma
BM	Bone Marrow
BMP	Bone Morphogenetic Protein
BFR	Bone Formation Rate
BMD	Bone Mineral Density
BV/TV	Bone Volume/Tissue Volume
BVF	Bone Volume Fraction
Ca-SGP	Sialoglycoprotein
CatK	Cathepsin K
CCL	Chemokine (C-C motif) Ligand
CFU	Colony forming unit
CONV-R	Conventionally Raised

Collα1	Collagen α1
CRT-THK-C	Cortical Thickness
Ct.Ar	Cortical Bone Area
Ct.Th	Cortical Bone Thickness
CTSK	Cathepsin K
CTX	C-terminal telopeptide
DT	Diphtheria Toxin
DEXA	Dual Energy X-ray Absorptiometry
ELISA	Enzyme-linked immunosorbent assay
FMO	Flavin-containing Monooxygenase
FOXP3	Forkhead box P3
FOS	Fructooligosaccharide
GC	Glucocorticoid
GC-MS	Gas Chromatography–Mass Spectrometry
GF	Germ Free
GH	Growth Hormone
GM	Gut microbiota
GIOP	Glucocorticoid Induced Osteoporosis
GIP	Gastric Inhibitory Polypeptide
GSH	Glutathione
HE	Haematoxylin and eosin
HFD	High Fat Diet
HPRT	Hypoxanthine-guanine Phosphoribosyl Transferase
ICP-MS	Inductively coupled plasma mass spectrometry
IL	Interleukin
IGF-1	Insulin-like Growth Factor 1
IGF1r	Insulin-like Growth Factor 1 receptor
IGFBP3	Insulin-like Growth Factor-Binding Protein 3
INF-γ	Interferon gamma
MAR	Mineral Apposition Rate

MDA	Malondialdehyde
MDY	High Molecular-weight Polymer
L. para	*Lactobacillus paracasei*
L. reuteri	*Lactobacillus reuteri*
LAL	Limulus Amoebocyte Lysate
LCN2	Lipocalin-2 Protein
LGG	Lactobacillus rhamnosus
NOD	Nucleotide Binding Oligomerization Domain
NF-κB	Nuclear Factor Kappa-light chain-enhancer of B cells
OCL	Osteoclast-like cells
OCN	Osteocalcin
OPG	Osteoprotegerin
OVX	Ovariectomy
OGTT	Oral Glucose Tolerance Test
PINP	N-terminal propeptide of type 1 procollagen
PPARγ	Peroxisome proliferator-activated receptor
PTH	Parathyroid Hormone
qRT-PCR	quantitative reverse transcription polymerase chain reaction
PQCT	Peripheral Quantitative CT
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RUNX2	Runt-related transcription factor 2
SAMP6	Senescence-Accelerated Mouse Prone 6
SCFA	Short-Chain Fatty Acid
SCD	Sickle cell disease
SFB	Segmented Filamentous Bacteria
SIRT	Sirtuin
SPARC	Osteonectin (also known as ON)
TAC	Total Antioxidant Capacity
Tb.N	Trabecular Bone Number
Tb.Sp	Trabecular Bone Spacing

Tb.Th	Trabecular Bone Thickness
TDF	Tenofovir Disoproxil Fumarate
TGF-β1	Transforming growth factor beta
TLR	Toll-Like Receptor
TRAP	Tartrate-Resistant Acid Phosphatase
TNF-α	Tumour Necrosis Factor α
Tt.Ar	Total Cross-Sectional Area
μCT	Micro-CT
VSEE	Val-Ser-Glu-Glu

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