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journal homepage: <https://www.sciopen.com/journal/2097-0765>Acidic *Lycium barbarum* polysaccharide as a promising candidate for preventing osteosarcopeniaAnni Yao^{a,1}, Xiaofei Liang^{a,1}, Zhengfei Wang^a, Daoping Sha^a, Xinlan Zhang^a, Mengqiu Liu^a, Liqiang Zhao^a, Yanan Liu^a, Sheng Guo^{a,b}, Fang Zhang^a, Chunjie Bao^{a,c,*}, Jialun Duan^{a,*}, Jin'ao Duan^{a,*}^a Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, National and Local Collaborative Engineering Center of Chinese Medicinal Resources Industrialization and Formulae Innovative Medicine, Jiangsu Province Key Laboratory for Functional Substances in Traditional Chinese Medicine Formulae and Innovative Drug Discovery, Nanjing University of Chinese Medicine, Nanjing 210023, China^b Key Laboratory of National Forestry and Grassland Administration on Goji, Yinchuan 750000, China^c School of Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, China

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ABSTRACT

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As the global population ages, the prevalence of osteosarcopenia—a combined condition of osteoporosis and sarcopenia—poses significant health challenges, leading to increased frailty, falls, and chronic disability in the elderly, especially for postmenopausal women. Nutritional interventions targeting age-associated physiological decline present promising strategies for managing these conditions. In this study, the preventive effects of total *Lycium barbarum* polysaccharide (LBP), acidic LBP (ALBP), and neutral LBP (NLBP) on osteosarcopenia were systematically evaluated using an ovariectomized (OVX) mouse model. Our findings showed that ALBP significantly improved physical performance, bone strength, and bone density, while enhancing muscle mass and fiber size, thereby effectively alleviating osteosarcopenia. Mechanistic investigations revealed that ALBP elevated the expression levels of osteoblast-associated genes such as *Alp*, *Colla1*, *Runx2*, *Opn*, upregulated key myogenic genes, including *Myh2*, *Myh4*, *Myh7*, while activating the phosphoinositide 3 kinase-protein kinase B (PI3K-AKT) signaling pathway. Dose-response relationship studies confirmed the efficacy of ALBP in preventing osteosarcopenia. Further analysis using gut microbiota sequencing, depletion models, and correlation analysis demonstrated that the preventive effects of ALBP are strongly correlated with the gut microbiota, including Bacteroidota, Firmicutes, Desulfobacterota, and Patescibacteria. These results highlight the potential of ALBP as a promising candidate for preventing osteosarcopenia and improving the well-being of the elderly.

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1. Introduction

With the accelerated aging of the global population, the prevalence of patients with multiple comorbidities is likewise increasing^[1]. Functional decline in the musculoskeletal system, comprising both bone and muscle, is common among elderly populations^[2-3]. This decline often precipitates osteosarcopenia,

a syndrome characterized by concurrent bone loss/osteoporosis and sarcopenia, that significantly diminishes daily functional capacity and quality of life in older adults^[4-6]. Osteosarcopenia has thus emerged as a growing public health concern^[7-8], with an increasing need for its prevention and management. Postmenopausal women are particularly vulnerable due to both aging and the decline in estrogen levels^[9-10].

Currently, clinical treatment options for osteoporosis include bisphosphonates and estrogen replacement therapy^[11-13]. However, there are currently few approved drugs to treat sarcopenia, which is predominantly managed through physical therapies, such as muscle strengthening, gait training, and protein supplementation^[14-16]. Therefore, the development of effective therapeutic or preventive interventions for osteosarcopenia is of significant scientific and clinical importance.

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Wolfberry is a medicinal and edible herb, and previous research has shown that water extracts of *Lycium barbarum* promote bone formation, with *L. barbarum* polysaccharide (LBP) identified as the primary active component^[17–21]. The present study aimed to isolate total LBP from *L. barbarum*, further separating them into acidic LBP (ALBP) and neutral LBP (NLBP). The ovariectomized (OVX) mouse model was established, and the comprehensive exercise ability, bone strength, bone mineral density (BMD), muscle weight, muscle fiber size, expression levels of related genes and proteins and their correlation with intestinal flora were evaluated after nutritional intervention with different types of LBP. This study provides a comparative evaluation of the effects of distinct LBP on osteosarcopenia and explores the potential mechanisms underlying these effects.

2. Materials and methods

2.1 Materials

Dried *L. barbarum* (wolfberry) was obtained from Ningxia Mingde TCM Decoction Pieces Co., Ltd. (Zhongwei, China), and estradiol valerate tablets were purchased from Bayer Healthcare Co., Ltd. Guangzhou Branch (Guangzhou, China). Total RNA rapid extraction reagent (including RNAPure) (#YFXM0011P) was purchased from Yfxbio (Nanjing, China). Quantitative reverse transcription polymerase chain reaction (RT-PCR) primers were synthesized by Tsingke Biotechnology Co., Ltd. (Nanjing, China). Rat C-telopeptide of type I collagen (CTX-I) enzyme-linked immunosorbent assay (ELISA) kit (CSB-E12782m) and rat N-terminal propeptide of type I collagen (PINP) ELISA kit (CSB-E12774r) were obtained from CUSABIO (<https://www.cusabio.com/>, Wuhan, China). Antibodies, including anti-GAPDH antibody (D16H11) XP (#5174T), anti-phosphoinositide 3 kinase (PI3K) p85 antibody (19H8) (#4228T), anti-protein kinase B (AKT, antibody (#9272S), anti-phospho-PI3K p85 antibody (Tyr458)/p55 (Tyr199) (#4228T), and anti-phospho-AKT (Ser473) (#9271T), were obtained from Cell Signaling Technology (Boston, USA). The horseradish peroxidase (HRP)-conjugated secondary antibody was purchased from Transgen (Beijing, China), and the GD muscle fixator (#G1111) was purchased from Servicebio (Wuhan, China).

2.2 Extraction of ALBP and NLBP

In this study, traditional hot water extraction was employed to isolate the ALBP and NLBP^[22]. Monosaccharides were initially removed using 80% ethanol, after which total LBP was obtained through water purification and precipitation. Anion exchange gradient elution was then performed using a DEAE-52 cellulose column (4.5 cm × 60 cm, 50 μm; Whatman Co., Ltd.). Pure water and 0.5 mol/L NaCl-elution fractions were collected, followed by freeze-drying after ultrafiltration membrane dialysis (Molecular Weight Cut Off, MWCO, 3 500 Da), yielding NLBP and ALBP, respectively.

2.3 Animals

Female BALB/c mice (6–8 weeks old, 18–20 g, purchased from Hangzhou Medical College Laboratory Animal Technology Co.,

Ltd.) were maintained in a specific pathogen-free (SPF) facility under standardized conditions, with a reversed 12-h light/dark cycle, for a one-week acclimatization period. Mice were weighed weekly throughout the study. This experiment was conducted with the approval and oversight of the Laboratory Animal Ethics Committee at Nanjing University of Chinese Medicine (ethics number: 202309A027).

Female SD rats (3 months old, 180–220 g, purchased from Shanghai Slack Laboratory Animal Technology Co., Ltd.) were maintained in a SPF facility under standardized conditions, with a reversed 12-h light/dark cycle, for a one-week acclimatization period. This experiment was conducted with the approval and oversight of the Laboratory Animal Ethics Committee at Nanjing University of Chinese Medicine (Ethics number: 202305A084).

2.4 Model construction and administration scheme

The mice were stratified into six experimental groups: sham-operation (Sham, $n = 8$), bilateral ovariectomy (OVX, model, $n = 8$), OVX mice treated with ALBP (ALBP, $n = 8$), OVX mice treated with NLBP (NLBP, $n = 8$), OVX mice treated with total LBP (total LBP, $n = 8$), and OVX mice administered estradiol valerate (EV, $n = 8$). EV served as the positive control in this study. OVX model was established as previously reported^[23]. Briefly, in the sham group, fat surrounding the ovaries was excised, while bilateral ovariectomy was performed on mice in the other groups. Each mouse received an intraperitoneal injection of 8 000 U of penicillin G sodium for three consecutive days to prevent postoperative infections. The ALBP, NLBP, and total LBP groups were administered 200 mg/kg of their respective treatments for 12 weeks, while mice in the EV group received 0.1 mg/kg of EV for the same duration. Mice in the Sham and model groups were treated with normal saline over the 12-week period.

2.5 Behavioral evaluation

Behavioral evaluation was investigated as previously reported^[24]. Following the treatment phase, the overall physical performance of the mice was assessed through a series of tests, including the forelimb grip strength test, pole climbing test, and wheel running test. The forelimb grip strength test measured the grip strength using a grip strength meter, while the pole climbing test evaluated the time required for the mice to descend from a vertically positioned wooden pole of approximately 1 meter. The running wheel experiment quantified the distance traveled by the mice in the running wheel over a 5 min period.

2.6 Biomechanical detection of tibia

The mouse tibia biomechanics was commissioned for testing at Sunpharma Co., Ltd.. An Instron E10000 universal material testing machine (Instron, USA) was utilized for mechanical testing of the tibial samples. During this assessment, the tibia was positioned between two supporting crossbars, with a loading rod applied centrally over a 6 mm span. All specimens were subjected to uniform loading at a rate of 3 mm/min until fracture occurred. The maximum load and deformation prior to fracture were recorded, and the parameters of

bending stiffness, Young's modulus, strain, and bending stress were subsequently calculated.

2.7 Micro-computed tomography analysis

Femurs from the mice were collected and scanned using Skyscan1276 X-ray microtomograph (micro-CT) software (Bruker, Germany) at a scanning resolution of 6.5 μm , with a voltage of 60 kV and a current of 200 μA . A 1 mm-thick region within the bone marrow cavity was selected to analyze trabecular bone distribution. The 3D image reconstruction was performed using N-Recon software, while 3D analysis was conducted with CT-AN software, which included assessments of BMD, bone volume fraction in total volume (BV/TV), structural model index (SMI), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp).

2.8 Muscle index evaluation

After mice were sacrificed, the hind limb muscles (including *soleus*, *tibialis anterior*, and *gastrocnemius* muscles) were collected and weighed using a BT125D analytical balance (Sartorius Scientific Instruments, Germany). Muscles were fixed, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (HE). Size and spacing of muscle fiber were subsequently analyzed using Slide Viewer software.

2.9 Section staining and immunohistochemistry

After mice were sacrificed, femurs were fixed in 4% paraformaldehyde for 24 h, followed by HE staining, alizarin red staining (G1038, Servicebio), or tartrate-resist-ant acid phosphatase (TRAP) immunohistochemical staining (G1050, Servicebio) for further analysis. Tissue samples from the heart, liver, spleen, lung, and kidney were similarly fixed for 24 h in 4% paraformaldehyde and subjected to HE staining.

2.10 RT-PCR analysis

Mouse muscle and bone marrow tissues were processed using an automatic tissue grinder (Tissuelyser-48, Shanghai Jingxin Technology) to isolate total RNA with TRIzol reagent, and RNA concentrations were measured using a microspectrophotometer (Denovix). cDNA synthesis was conducted with the TransScript one-step gDNA removal and cDNA synthesis SuperMix kit, followed by cDNA amplification using specific primers and PerfectStart Green qPCR SuperMix in a real-time PCR system (Applied Biosystems, USA). Relative gene transcription levels were normalized to *GAPDH* expression and calculated using the $2^{-\Delta\Delta C_t}$ method. All primer sequences were designed based on data from the National Center for Biotechnology Information (NCBI) (Table S1).

2.11 Detection of serum levels of bone-related markers

Serum estradiol 2 (E2) and the PINP were quantified using ELISA kits (CSB-E05109, CSB-E12775; Cusabio, China, <https://www.cusabio.com/>) according to the manufacturer's protocol, with absorbance readings obtained at 450 nm using a multifunctional microplate reader (Perkin Elmer, USA).

2.12 Western blot analysis

Western blot analysis was conducted based on established protocols^[25]. In brief, muscle protein was extracted using RIPA buffer (Beyotime, Shanghai, China) supplemented with protease and phosphatase inhibitors (Beyotime, Shanghai, China). Protein concentrations were determined using a BCA assay kit (Beyotime, Shanghai, China), and samples were then mixed with $5 \times$ loading buffer and boiled for 10 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Darmstadt, Germany), and blocked in Tris buffered saline with Tween-20 (TBST) containing 5% BSA. Membranes were incubated overnight at 4 °C with primary antibodies, including anti-GAPDH (D16H11 XP), PI3K p85 (19H8), AKT, p-PI3K p85 (Tyr458)/p55 (Tyr199), and p-AKT (Ser473). Following TBST washes, membranes were incubated for 1 h at 25 °C with an HRP-conjugated secondary antibody and then visualized on a ChemiDoc XRS+ system (Bio-Rad, USA).

2.13 Intestinal bacteria detection

Mice were fasted for 24 h prior to euthanasia, and fecal samples were collected and stored at $-80\text{ }^{\circ}\text{C}$ for subsequent analysis. Microbiome profiling was performed by Meggie Biotechnology Co., Ltd., and data analyses were conducted through the "Megi Bioscience Service" network.

2.14 Micro-computed tomography analysis of rat femur

Same as in Section 2.7.

2.15 Section staining of rat femur

After rats were sacrificed, femurs were fixed in 4% paraformaldehyde for 24 h, followed by HE staining, Goldner staining (G1064, Hubei, China).

2.16 Detection of serum bone turnover related factors in rats

CTX-I and the PINP were quantified using ELISA kits according to the manufacturer's protocol, with absorbance readings obtained at 450 nm using a multifunctional microplate reader (PerkinElmer, USA).

2.17 Dose-response relationship studies and gut microbiota depletion models establishment

Mice were divided into six experimental groups: Sham ($n = 8$), model ($n = 8$), OVX mice treated with low-dose ALBP (ALBP-L, 50 mg/kg, $n = 8$), OVX mice treated with medium-dose ALBP (ALBP-M, 100 mg/kg, $n = 8$), OVX mice treated with high-dose ALBP (ALBP-H, 200 mg/kg, $n = 8$), OVX mice treated with high-dose ALBP and an antibiotic cocktail (ALBP-H + Abx, 200 mg/kg, $n = 8$). The antibiotic cocktail consisted of metronidazole (1 g/L), ampicillin (1 g/L), neomycin sulfate (1 g/L), and vancomycin hydrochloride (500 mg/L). To assess the impact

of gut microbiota depletion, mice in the ALBP-H + Abx group received the antibiotic cocktail as a substitute for drinking water over a 14-day period.

2.18 Statistical analysis

Stained tissue sections were quantified using SlideViewer 2.6 software, Western blot images were quantified with ImageJ software, and intestinal microbiome analysis were performed using the online platform of Majorbio Cloud Platform (<https://www.majorbio.com>). Correlation analyses between intestinal microbiota and osteosarcopenia parameters were conducted with SPSS software. Statistical analyses were performed using Prism 9.5.0 (GraphPad, La Jolla, CA, USA). One-way ANOVA was used for multiple group comparisons, while Student's *t*-test was employed for two-group comparisons.

3. Results

3.1 ALBP improved the comprehensive physical performance and bone strength of OVX mice

ALBP and NLBP were extracted from *Fructus Lycii* and further isolated by DEAE chromatography. The structural characterization, as detailed in our previous study^[25], determined their molecular weights as 37.4 kDa for ALBP and 83.4 kDa for NLBP. Both polysaccharides comprised mannose (Man), rhamnose (Rha), gluconic acid (GlcA), galacturonic acid (GalA), glucose (Glc), galactose (Gal), and arabinose (Ara). The monosaccharide distribution for ALBP was 0.69% Man, 4.61% Rha, 1.24% GlcA, 76.18% GalA, 0.55% Glc, 6.08% Gal, and 10.64% Ara. Similarly, the proportional distribution for NLBP was 1.75% Man, 3.29% Rha, 2.11% GlcA, 3.16% GalA,

16.83% Glc, 29.35% Gal, and 43.51% Ara, reflecting their distinct structural compositions.

To evaluate the protective effects of LBP on bone homeostasis, an OVX mouse model was established. Following 12 weeks of intragastric administration, mice underwent a comprehensive behavioral assessment to evaluate physical performance. Tibial, femoral, and serum analyses were performed to assess the impact on osteoporosis, while *soleus*, *tibialis anterior*, and *gastrocnemius* muscles were collected to evaluate sarcopenia improvements. Additionally, fecal samples were analyzed to investigate shifts in gut microbiota and their correlation with osteoporosis and sarcopenia (Fig. 1A).

Behavioral assessment indicated a significant decline in motor function in the model group compared to the sham group. Compared to the model group, in the ALBP-treated group, grip strength increased by 1.51-fold, climbing time decreased by 2.78-fold, and running distance increased by 4.16-fold, significantly outperforming both the NLBP and total LBP groups (Figs. 1B–D).

Tibial biomechanical testing revealed that, relative to the sham group, the model group showed substantial reductions in maximum load, maximum distortion, bending stiffness, bending stress, and Young's modulus, alongside a 54.67% increase in strain (Figs. 1E–J). These findings suggest that tibial bone fragility was elevated in the model group. In contrast, ALBP treatment significantly improved bone strength, with a 18% increase in maximum load, a 67% increase in maximum distortion, a 35% increase in bending stiffness, a 60% increase in bending stress, a 103% increase in Young's modulus, and a 34% reduction in strain. The NLBP group demonstrated significant improvement only in maximum load. Analysis of the force-distortion curve indicated that, compared to the Sham group, the OVX group had a lower maximum force tolerance, whereas ALBP administration enhanced femoral load tolerance (Fig. S1).

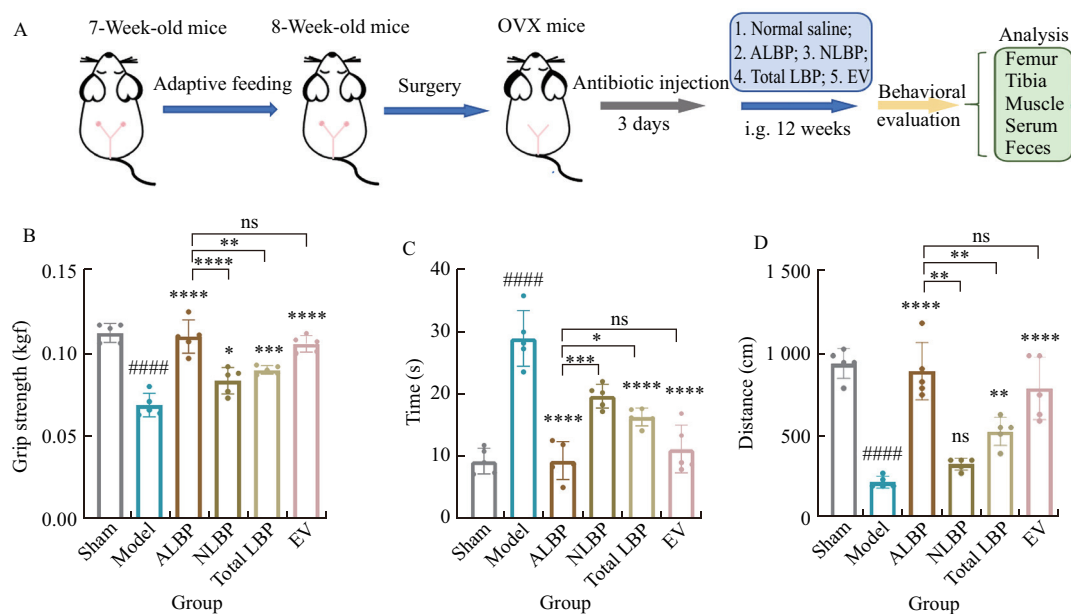


Fig. 1 ALBP improved the comprehensive physical performance and bone strength of OVX mice. (A) Experimental scheme of OVX mouse model establishing and administration. Behavioral assessment of mouse (B) grip strength, (C) time of pole climbing, and (D) movement distance of 5 min in the running wheel. Tibia three-point bending experiment analysis of (E) maximum load, (F) maximum distortion, (G) bending stiffness, (H) bending stress, (I) Young's modulus, and (J) strain. Data are expressed as mean $\pm$ standard deviation ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ represent treated groups vs. Model group. ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ represent Model group vs. Sham group. ns, no significance with model group, NS, no significance with Sham group. The same as below.

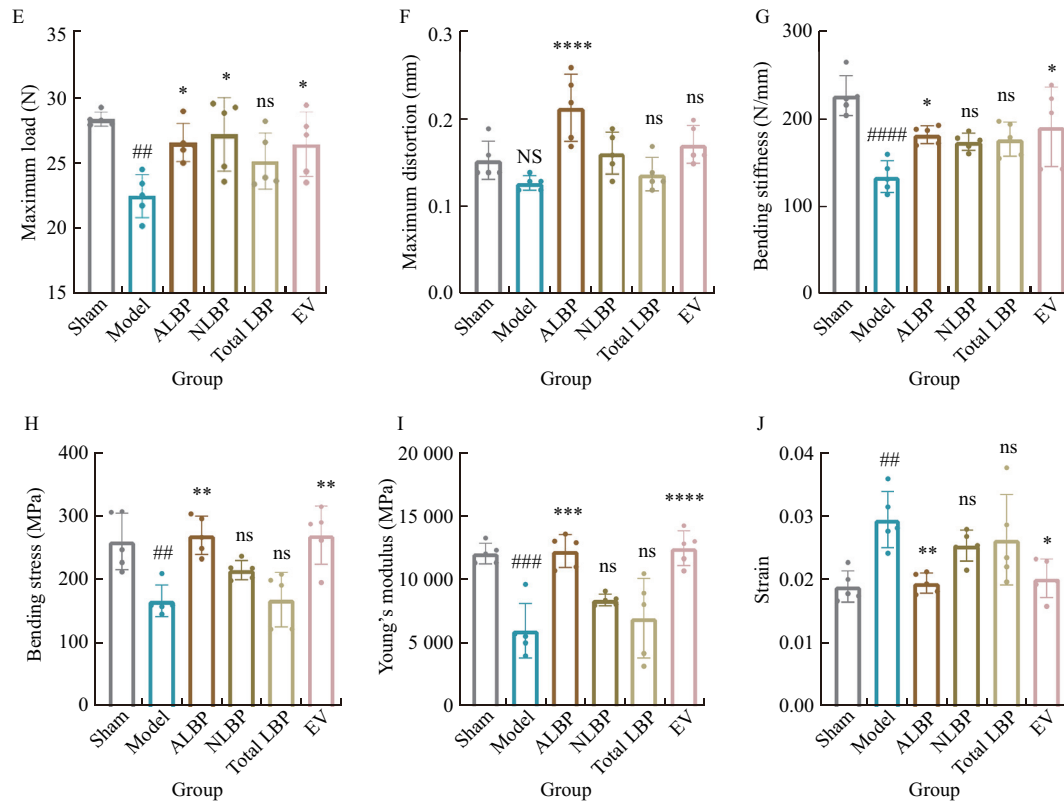


Fig. 1 (Continued)

3.2 ALBP improved osteoporosis by increasing bone density in OVX mice

To investigate the impact of LBP on bone structure changes in mice, femoral specimens underwent micro-CT scanning, with the resulting bone microarchitecture illustrated in Figs. 2A–C. In the model group, a significant reduction in the number of bone trabeculae was observed, alongside the presence of large cavities devoid of bone connection, indicating pronounced bone resorption and substantial micro-architectural disruption. In contrast, the EV group exhibited notable improvements in bone structure, characterized by uniform trabecular thickness and compact arrangements. Notably, the ALBP group displayed uniform trabecular thickness, enhanced continuity between trabeculae, and an increase in junction cavities, indicative of a more normal bone microstructure. Key parameters, including BV/TV, BV, Tb.N, Tb.Sp, SMI, and BMD, were significantly enhanced. Conversely, the NLBP group showed no notable improvements across these metrics, while the total LBP group exhibited limited improvements in BV/TV, Tb.N, and SMI, likely attributed to the presence of ALBP within the total LBP formulation (Figs. 2D–I). These findings suggest that ALBP is the primary active component of LBP exerting prevent osteoporotic effects.

Histological analysis via HE staining revealed abnormal trabecular structures in the model group, characterized by trabecular fractures and reduced bone marrow cavity and cell density. Both the EV and ALBP groups demonstrated improvements in trabecular integrity, with reductions in bone marrow cavity area and increases in bone marrow cell counts. No significant enhancements were noted in the NLBP and total LBP groups (Fig. 3A). Alizarin red staining clearly depicted reductions in the mineralized bone area in the OVX group, while both the EV and ALBP groups exhibited significant increases in mineralized

bone area among the osteoporosis-affected rats (Fig. 3B). TRAP staining highlighted a marked increase in osteoclast numbers in the model group, which significantly decreased in the EV and ALBP groups, indicating a restoration of osteoporotic conditions (Fig. 3C). Thus, ALBP appears to ameliorate osteoporosis by enhancing bone density in mice.

After 12 weeks of modeling, OVX mice exhibited significant weight gain (Fig. S2A). Furthermore, uterine morphology and quality were assessed across all groups, revealing that the ALBP group significantly mitigated uterine atrophy, suggesting that ALBP may enhance estrogen activity to combat osteoporosis (Figs. S2B–C). Existing literature supports the notion that estrogen promotes bone tissue growth and maturation, inhibits bone resorption, and effectively sustains bone health^[13]. Organ histological evaluations indicated no apparent safety concerns across the various treatment groups (Fig. S2D).

Additionally, the osteosarcopenia protectiveness of ALBP was also investigated in OVX rat models. Our finding demonstrated ALBP contributed to the increase of BMD (Figs. S3A–E), the enhancement of osteoblast/osteoclast ratio, the improvement of the bone microenvironment (Figs. S3F–G), reduction of CTX-1 levels, and elevated PINP levels in OVX rat models (Figs. S3H–I).

Collectively, these findings affirm that ALBP effectively improves osteoporosis in OVX mice, while the beneficial effects of NLBP and total LBP appear to be limited.

3.3 ALBP improved muscle atrophy by increasing muscle mass

Sarcopenia is a condition marked by reductions in muscle mass, a decreased number of muscle fibers, and expanded muscle

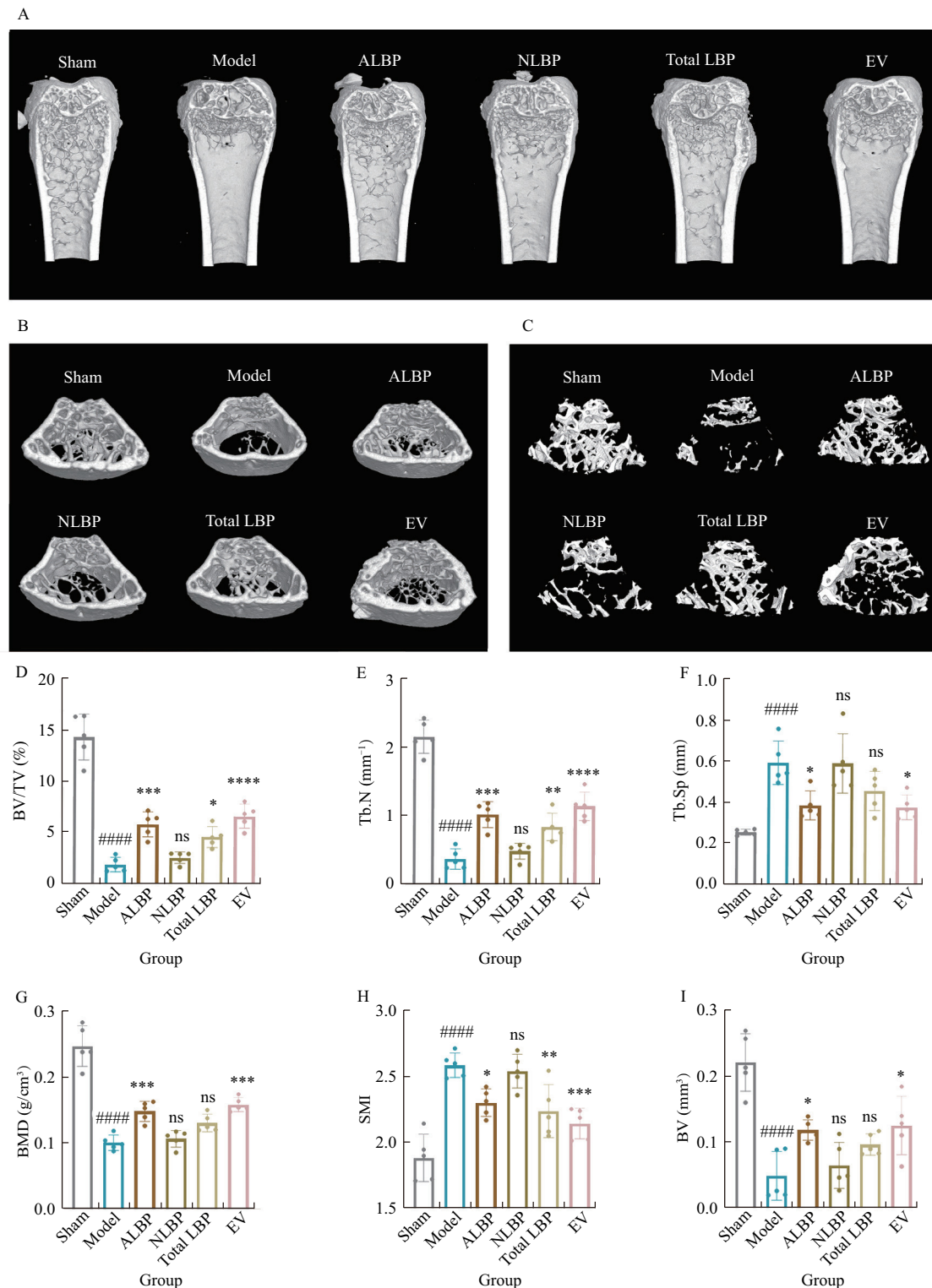


Fig. 2 ALBP improved osteoporosis by increasing bone density in OVX mice. (A) CT graphs of femur from longitudinal section. (B) CT graphs of femur from transverse section. (C) CT graphs of trabecular bone. CT analysis of (D) BV/TV, (E) Tb.N, (F) Tb.Sp, (G) BMD, (H) SMI, and (I) BV.

fiber spacing, as previously reported^[26]. Muscle mass and muscle atrophy were assessed using two primary parameters. First, muscle weight was evaluated via muscle index. Compared with the sham group, the muscle indices for the *soleus*, *tibialis anterior*, and *gastrocnemius* muscles were significantly reduced in the model group, suggesting a notable presence of muscle loss in osteoporotic mice, consistent with the previously observed decline in motor

abilities in this group. In contrast, the ALBP group demonstrated a significant increase in muscle weight, indicating that ALBP may serve as the primary active component of LBP in mitigating muscle loss (Figs. 4A–C).

Subsequently, muscle atrophy was evaluated based on muscle fiber spacing and size. Histological examination via HE staining showed that,

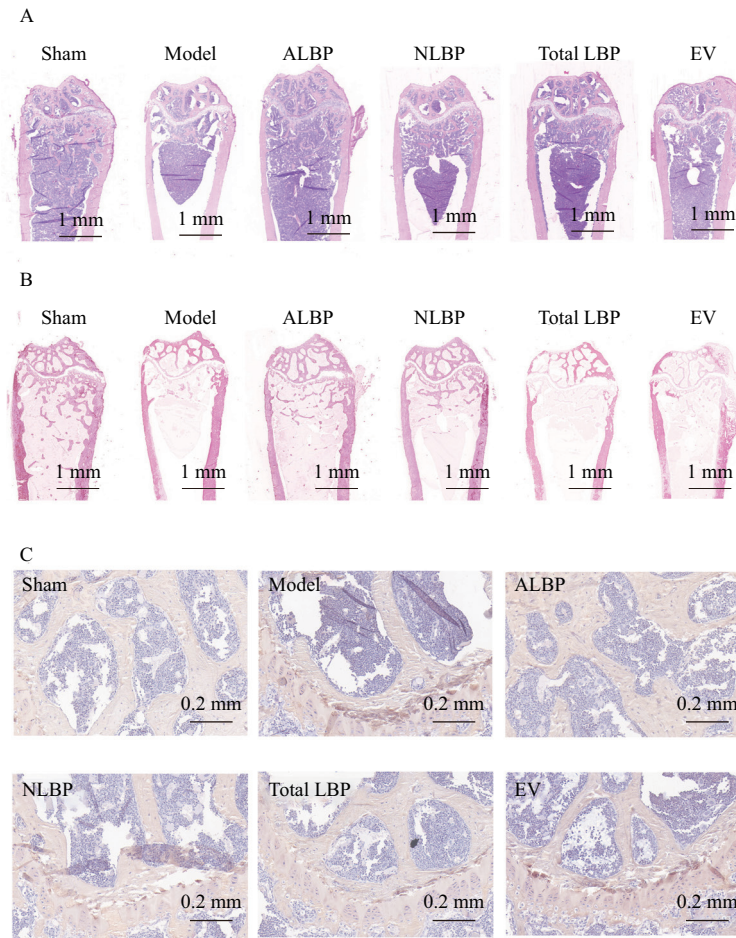


Fig. 3 Histological examination and immunohistochemical analysis of femur. (A) HE staining of femur in mice across different treatment groups. (B) Alizarin red staining of femur in mice across different treatment groups. (C) TRAP immunohistochemical analysis of femur in mice across different treatment groups.

relative to the sham-operated group, muscle fiber size and inter-fiber spacing were markedly reduced in the model group, confirming the presence of muscle atrophy. The ALBP group exhibited an 82.1%, 78.6%, and 79.1% decrease in muscle fiber spacing and a corresponding 20%, 110%, and 41.6% increase in muscle size across evaluated muscles. However, improvements in muscle fiber size were observed solely in the gastrocnemius muscle ($P < 0.01$; Figs. 4D–J), suggesting that ALBP may promote muscle cell proliferation with limited effects on slow-twitch muscle fibers.

3.4 Potential mechanism of ALBP to improve osteoporosis and muscle loss

Given the beneficial effects of ALBP on osteoporosis and muscle atrophy, we further investigated the underlying action mechanisms. To assess gene expression related to osteoblast activity in the femur and muscle fiber composition in skeletal muscle, RT-PCR was conducted. In the model group, expression levels of osteoblast-associated genes *Alp*, *Colla1*, *Runx2*, and *Opn* were markedly reduced. Treatment with various types of LBP resulted in increased expression of these genes across administration groups (Figs. 5A–D), with the ALBP group demonstrating substantial upregulation of *Alp*, *Colla1*, *Runx2*, and *Opn* by 8.9, 36.1, 3.9, and 23.9 times, respectively.

In the muscle tissue of model mice, expression levels of genes encoding muscle fiber types—*Myh2*, *Myh4*, and *Myh7*—were elevated in the *tibialis anterior*, *gastrocnemius*, and *soleus* muscles of the ALBP group (Figs. 5E–G). Further analysis using serum ELISA assessed bone turnover markers and estrogen levels, revealing significantly increased serum PINP and E2 in the ALBP group ($P < 0.05$; Figs. 5H–I). Sarcopenia is often driven by an imbalance in protein synthesis and degradation, and the PI3K/AKT pathway is a crucial pathway involved in this process^[27]. Western blot analysis of the *tibialis anterior* muscle showed that ALBP treatment significantly upregulated the phosphorylation levels of PI3K and AKT ($P < 0.01$; Figs. 5J–L and S4), indicating that ALBP may activate the PI3K/AKT pathway, thereby reducing muscle protein degradation. Fig. 5M provides a summary of the mechanisms by which ALBP exerts protective effects against osteoporosis and sarcopenia. Collectively, these results suggest that elevated expression of PINP and E2 and enhanced phosphorylation of PI3K/AKT play critical roles in the protective effects of ALBP on the skeletal and muscular systems in OVX mice.

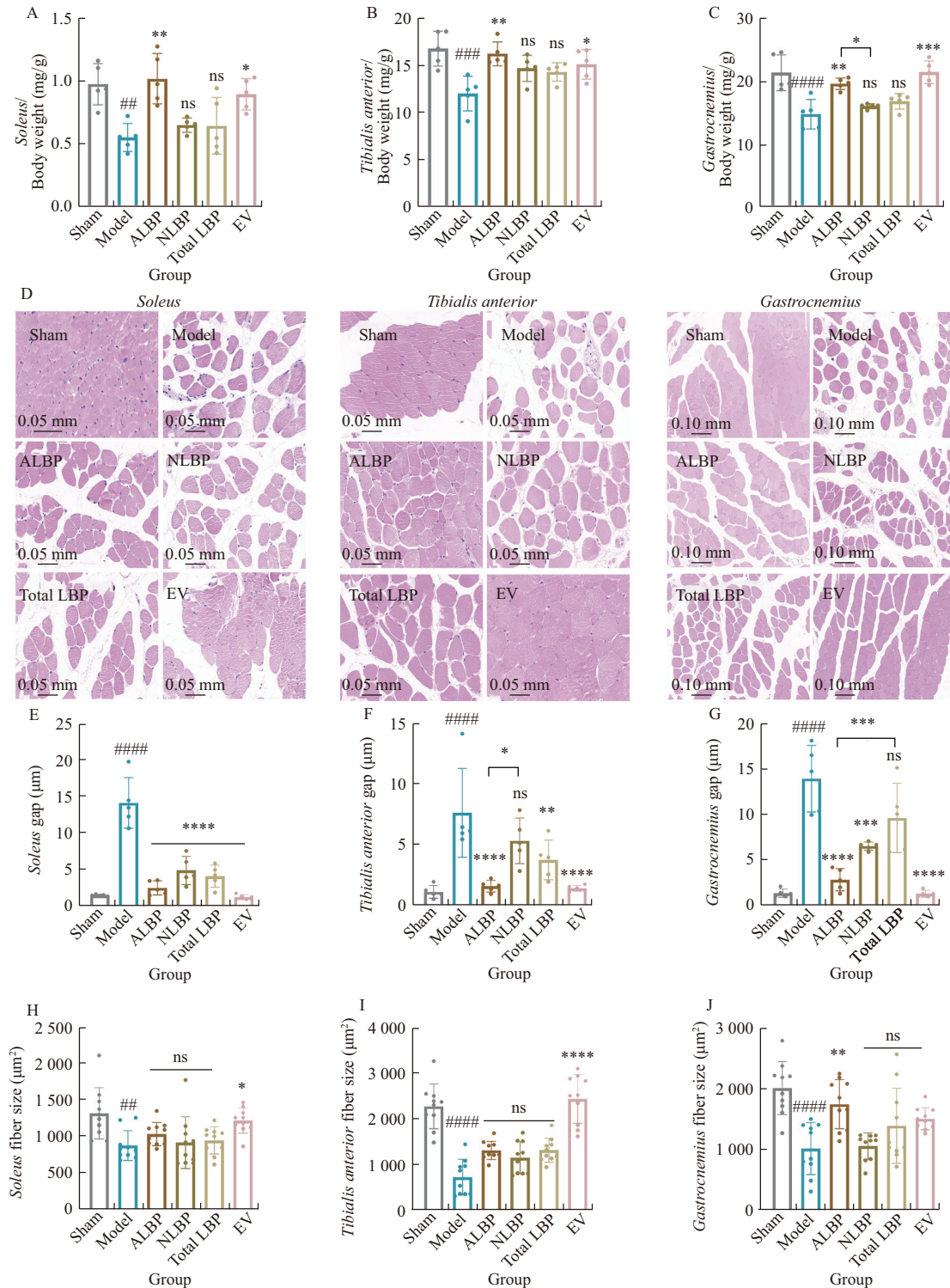


Fig. 4 ALBP improved muscle atrophy by increasing muscle mass. Muscle index of (A) soleus, (B) tibialis anterior and (C) gastrocnemius muscles ($n = 5$). (D) Histological examination of soleus, tibialis anterior, gastrocnemius muscles ($n = 5$). Muscle fiber gap analysis of (E) soleus, (F) tibialis anterior and (G) gastrocnemius muscles. Muscle fiber size analysis of (H) soleus, (I) tibialis anterior and (J) gastrocnemius muscles ($n = 10$).

3.5 ALBP improved the distribution of intestinal bacteria in OVX mice

Polysaccharides serve as a significant energy source for gut microbes, allowing for the metabolism of various complex carbohydrates and contributing to intestinal homeostasis by producing metabolites such as short-chain fatty acids, which are beneficial for host

health^[28-30]. To explore whether gut microbiota modulation contributes to the preventive effects against osteosarcopenia of LBP, fecal samples from mice were collected for gut microbiota analysis.

Alpha diversity is a key measure of the diversity, richness, and stability of bacterial communities within an individual. Analysis of extensive sample data indicated that, relative to the sham group, the diversity and stability of gut microbiota were significantly reduced in

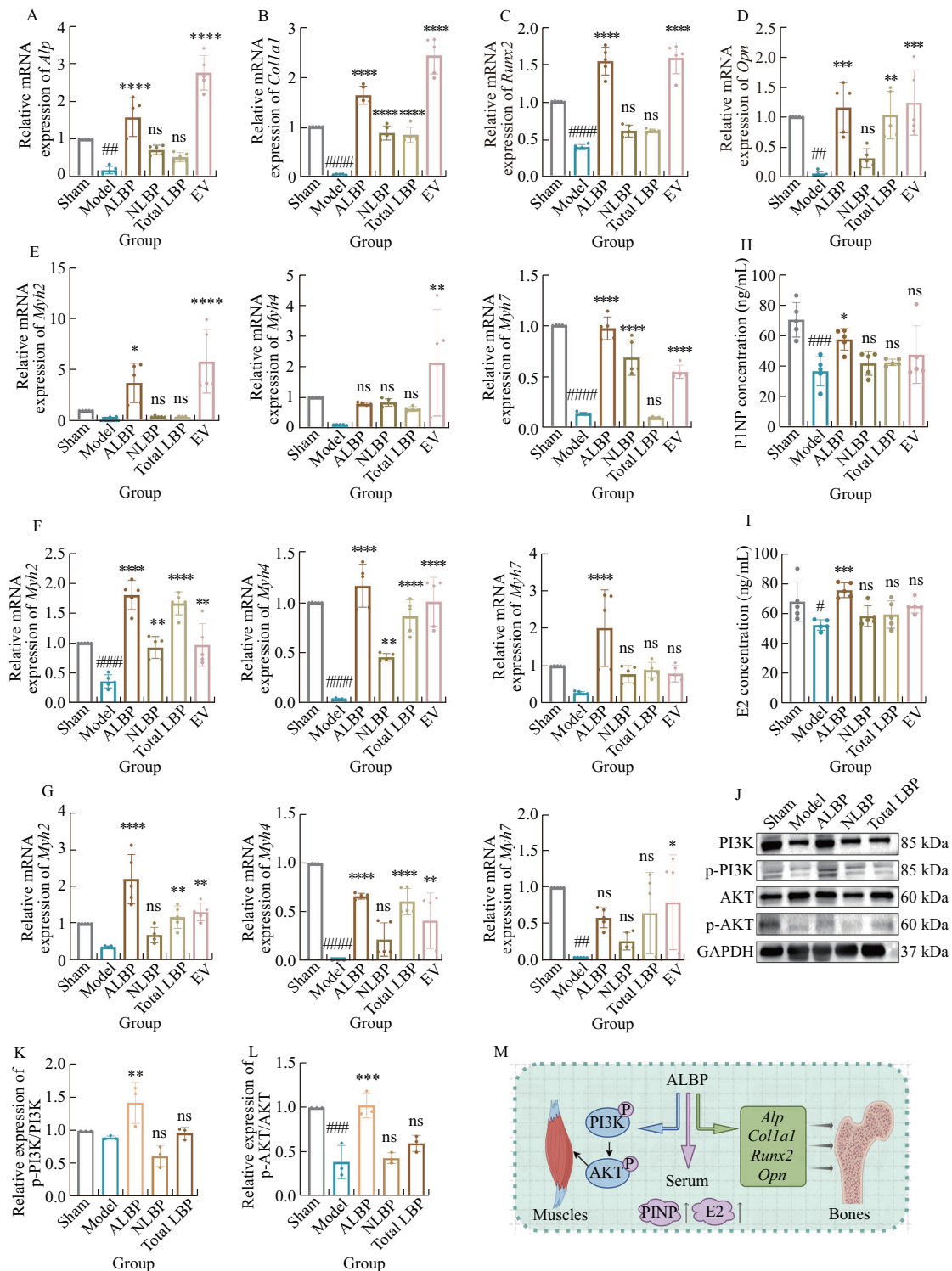


Fig. 5 Potential mechanisms of ALBP in improving osteoporosis and muscle atrophy. Expression levels of osteogenic genes (A) *Alp*, (B) *Col1a1*, (C) *Runx2*, (D) *Opn* across different treatment groups in bone marrow. Expression levels of muscle fiber related genes *Myh2*, *Myh4* and *Myh7* in (E) soleus, (F) tibialis anterior and (G) gastrocnemius muscles. Serum concentration of (H) PINP and (I) E2. (J–L) Western blot analysis of the protein expression in PI3K-AKT pathway. (M) Potential mechanism illustration of ALBP to regulate bones and skeletal muscles.

the model group, with substantial improvement observed in the ALBP group. This suggests that ALBP effectively enhances the richness and evenness of intestinal microbiota in mice (Figs. 6A–E).

Beta diversity analysis based on principal coordinates analysis (PCoA) showed a significant separation of the gut microbiota

between the model group and the sham group. Notably, the microbial community profile in the ALBP-treated group shifted closer to that of the sham group, suggesting that ALBP administration may partially restore gut microbiota composition toward a healthy state (Fig. 6F). This suggests that ALBP administration promotes a gut microbial

profile closer to the healthy, sham group. Venn diagram analysis further illustrated a 44.5% reduction in unique bacterial OTU number in the model group, confirming the decline in microbial diversity associated with osteoporosis (Fig. 6G).

Sequencing of fecal samples revealed that the predominant bacterial phyla across groups included Firmicutes, Bacteroidota, Patescibacteria, Campilobacterota, and Desulfobacterota (Fig. 6H). At the genus level, norank_f_Muribaculaceae, Lachnospiraceae_NK4A136_group, and *Lactobacillus* were dominant (Figs. 6I–J). *Lactobacillus*, known for its role in dietary fiber polysaccharide degradation and fermentation, supports the growth of various beneficial bacteria. The relative abundance of *Lactobacillus* significantly decreased in the

model group but was reversed by a 2.17-fold increase in the ALBP group. Additionally, Compared with the model group, the abundance of Lachnospiraceae_NK4A136_group in the intestinal flora of mice in the ALBP group and the total LBP group was significantly increased. Previous studies have shown that Lachnospiraceae_NK4A136_group contributes to elevated short-chain fatty acids (SCFAs), which may benefit bone and muscle health by mitigating osteoporosis and muscle atrophy^[31].

Species composition differences among the groups were further examined. Linear discriminant analysis effect size (LEfSe) multilevel discriminant analysis (Fig. 6K) identified distinct taxa across groups,

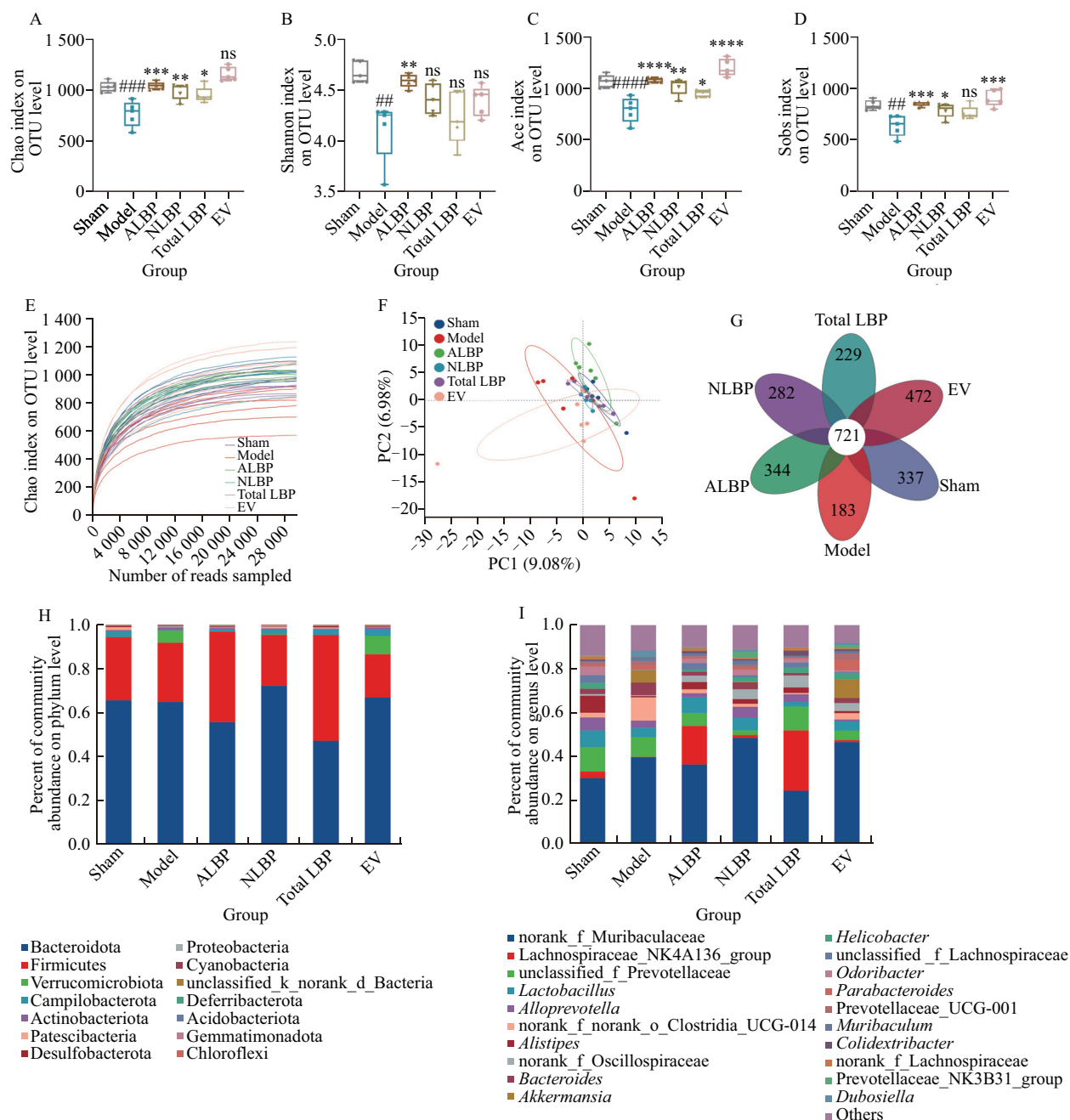


Fig. 6 ALBP improved the distribution of intestinal bacteria in OVX mice. α -Diversity analysis of (A) Chao index, (B) Shannon index, (C) Ace index, (D) Sobs index of intestinal bacteria across different treatment groups. (E) Dilution curve chart. (F) β -Diversity analysis of intestinal bacteria. (G) Venn diagram of OTUs per sample. Intestinal bacterial species composition at (H) phylum and (I–J) genus levels. (K) LEfSe multilevel discriminant analysis.

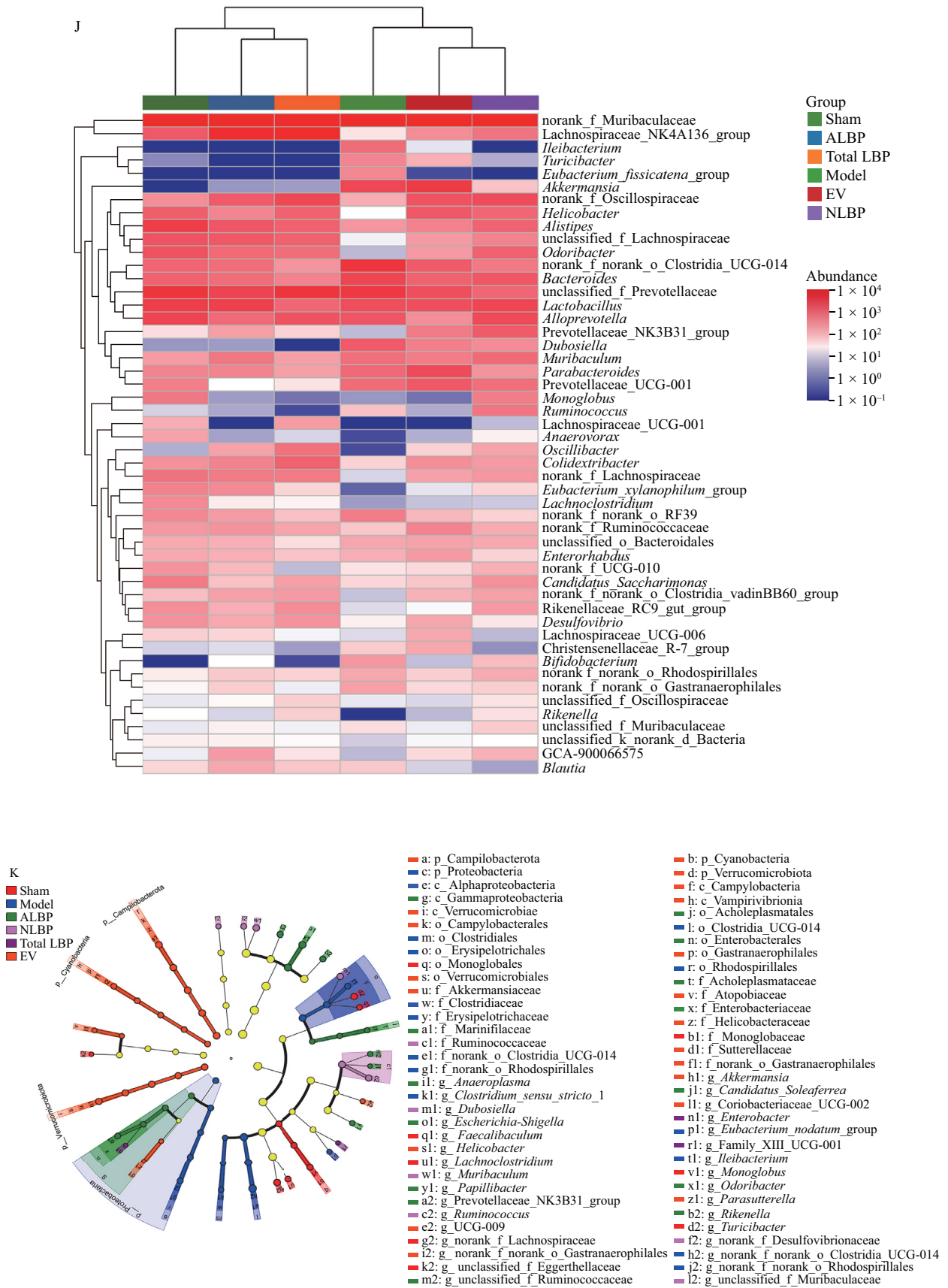


Fig. 6 (Continued)

with notable bacterial genera being *g_norank_f_Lachnospiraceae* and *g_Turicibacter* in the sham group, *o_Clostridia_UCG-014* and *o_Erysipelotrichales* in the model group, and *g_Odoribacter*, *f_Marinifilaceae*, and *g_Prevotellaceae_NK3B31_group* in the ALBP group. Among them, the increase of *Prevotella* abundance in the ALBP treatment group was the same as in previous studies^[23]. These findings provide a framework for understanding potential therapeutic targets for osteoporosis and muscle wasting. Collectively, the data support that ALBP may ameliorate osteosarcopenia by modulating gut microbiota composition in mice.

3.6 ALBP exhibited dose-dependent manner in preventing osteosarcopenia

After 12 weeks of intragastric administration, a comprehensive evaluation of physical performance of mice was conducted. BMD and bone strength were assessed in the femur and tibia, while muscle weight and fiber structure were evaluated in the *soleus*, *tibialis anterior*, and *gastrocnemius* muscles. This enabled an in-depth exploration of the dose-dependent effects of ALBP in preventing osteosarcopenia. Behavioral assessments revealed that, compared to the model group, all ALBP treatment groups (low, medium, and high doses) exhibited a progressive improvement in exercise capacity with increasing ALBP concentration. However, it is noteworthy that, despite receiving the high dose of ALBP, the ALBP-H + Abx group exhibited significantly reduced physical performance compared to the ALBP-H group. (Figs. 7A–C).

To evaluate the impact of ALBP on bone structure, we performed micro-CT scanning of the femur and biomechanical testing of the tibia. The results indicated that all ALBP treatment groups (low, medium, and high doses) significantly improved BMD and bone strength compared to the model group, with a dose-dependent response. However, the ALBP-H + Abx group exhibited less improvement in BMD and bone strength compared to the ALBP-H group (Figs. 7D–J).

Evaluation of the *soleus*, *tibialis anterior*, and *gastrocnemius* muscles revealed a dose-dependent increase in muscle weight with escalating concentrations of ALBP, ultimately reaching levels comparable to those observed in healthy mice (Figs. 8A–C).

Histological examination using HE staining demonstrated that ALBP exhibited a dose-dependent effect on improving muscle fiber size and reducing the spacing between muscle fibers (Figs. 8D–L). However, the ALBP-H + Abx group displayed reduced efficacy in alleviating muscle loss compared to the ALBP group, suggesting the critical role of gut microbiota in the development of osteosarcopenia.

In conclusion, ALBP exhibited a dose-dependent effect in enhancing overall physical performance, promoting bone health, and enhancing muscle mass and fiber size in mice. Its therapeutic efficacy was closely associated with modulation of the gut microbiota, highlighting the potential role of the microbiome in mediating the benefits of ALBP.

3.7 Correlation analysis between intestinal bacteria and osteosarcopenia

To investigate the potential role of intestinal microbial composition in the prevent osteosarcopenic effects of ALBP, we conducted Pearson correlation analysis to assess correlation between intestinal bacteria communities and both osteoporosis and sarcopenia. The results indicated that the efficacy of ALBP in preventing osteoporosis was significantly correlated with several intestinal microorganisms, including Bacteroidota, Firmicutes, Desulfobacterota, and Patescibacteria (Fig. 9A). Similarly, the ameliorative effects on muscle loss were notably correlated with Bacteroidota, Firmicutes, Cyanobacteria, Desulfobacterota, and Patescibacteria (Fig. 9B). These findings underscore the intricate relationship between gut microbiota and the pathophysiology of osteoporosis and sarcopenia in the context of ALBP treatment.

4. Discussion

A recent study reported a 50% prevalence of osteoporosis—an age-related chronic disease—among postmenopausal women^[5]. Notably, the coexistence of osteoporosis and sarcopenia significantly elevates fracture incidence, mortality rates and social costs in older adults, adversely impacting their quality of life^[32–33]. Furthermore, evidence suggests overlapping pathophysiological mechanisms between sarcopenia and osteoporosis, indicating the potential for a unified treatment approach for both conditions. This highlights the

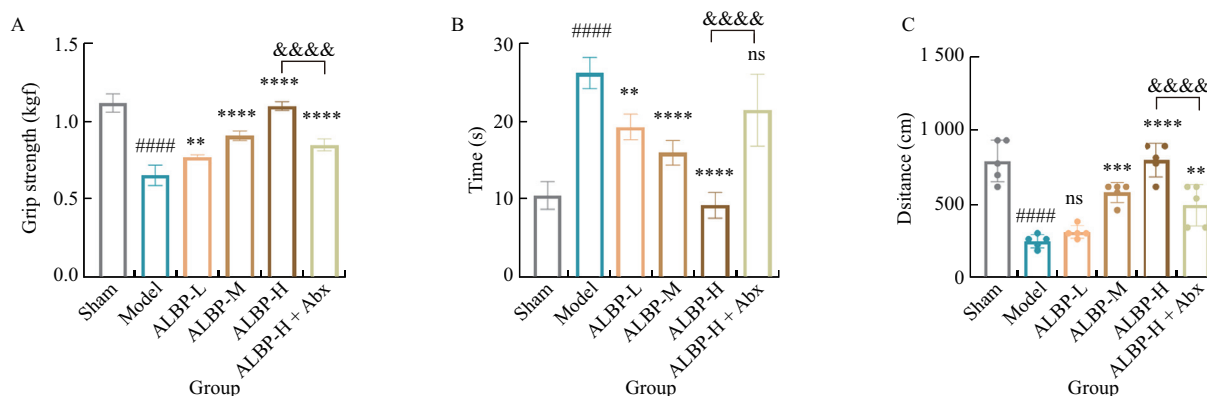


Fig. 7 ALBP exhibited dose-dependent manner in improving comprehensive physical performance and increasing bone health in mice. Behavioral assessment of mouse (A) grip strength test, (B) time of pole climbing, and (C) movement distance of 5 min in the running wheel ($n = 5$). (D) CT graphs of femur from longitudinal section, transverse section and trabecular bone. CT analysis of (E) BV/TV, (F) Tb.N and (G) Tb.Sp. Effects of each administration group on bone strength of OVX mice (H) Maximum distortion, (I) Bending energy, (J) Strain. &&&& $P < 0.0001$ represent ALBP vs. ALBP + Abx groups. The same as below.

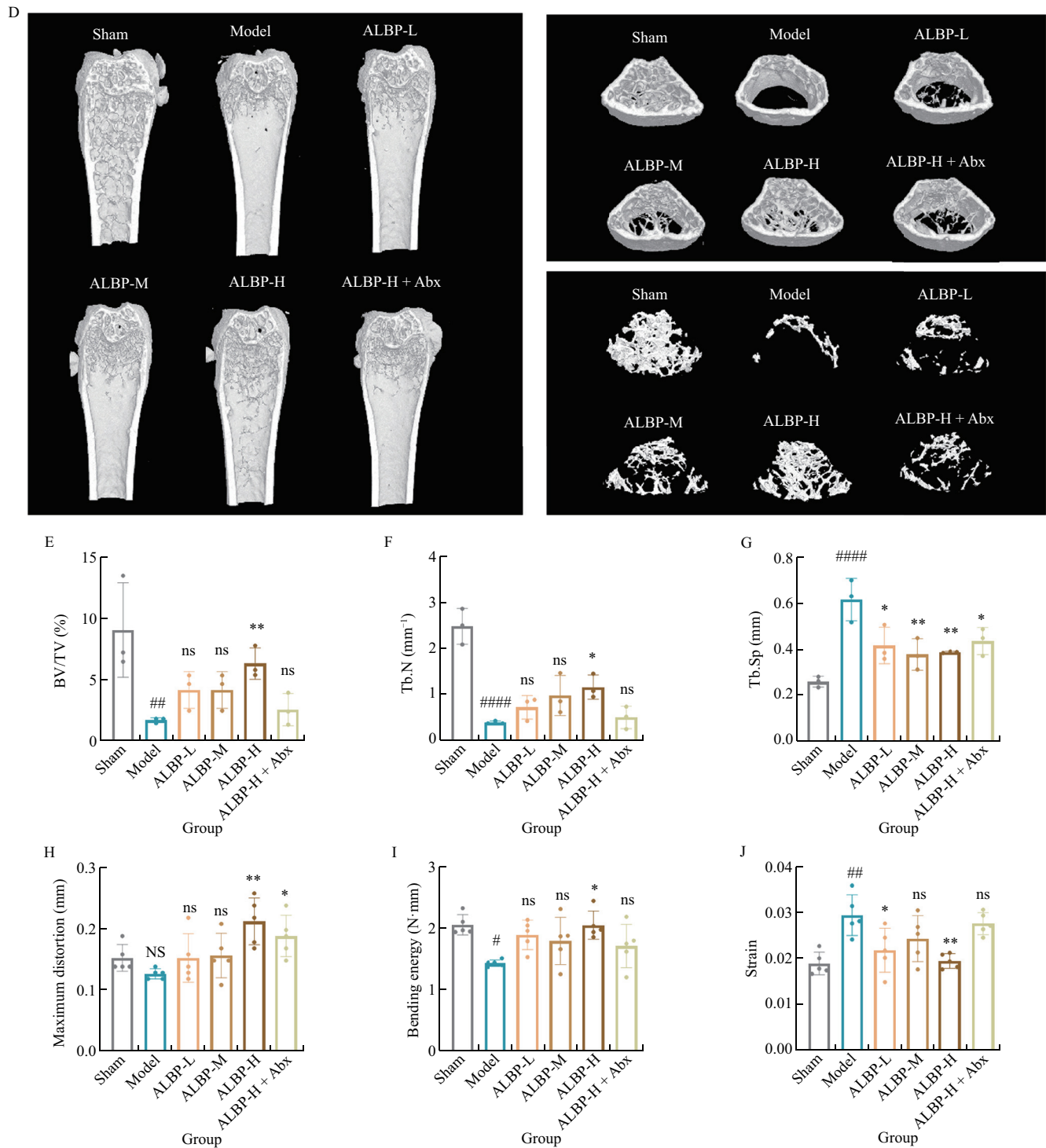


Fig. 7 (Continued)

importance of identifying therapeutic agents that can effectively address both osteoporosis and sarcopenia.

In line with the previous findings^[34–35], which demonstrated the promotion of osteoblast differentiation of LBP in MC3T3-E1 cells, our results from *in vivo* OVX mice models corroborate this effect. In this study, we isolated ALBP and NLBP from LBP and evaluated their effects through micro-CT and biomechanical assessments. We observed that ALBP promoted osteoblast differentiation while

inhibiting osteoclast differentiation (Fig. 3), leading to enhanced trabecular bone density and bone strength (Figs. 1–2). Due to their resistance to direct digestion and absorption in the gastrointestinal tract, polysaccharides reach the colon where they are fermented into bioactive compounds like SCFAs, thereby exerting their physiological effects^[36]. For instance, *Flammulina velutipes* polysaccharide has been shown to regulate the intestinal environment and enhance immune function by increasing the relative abundance of SCFA-

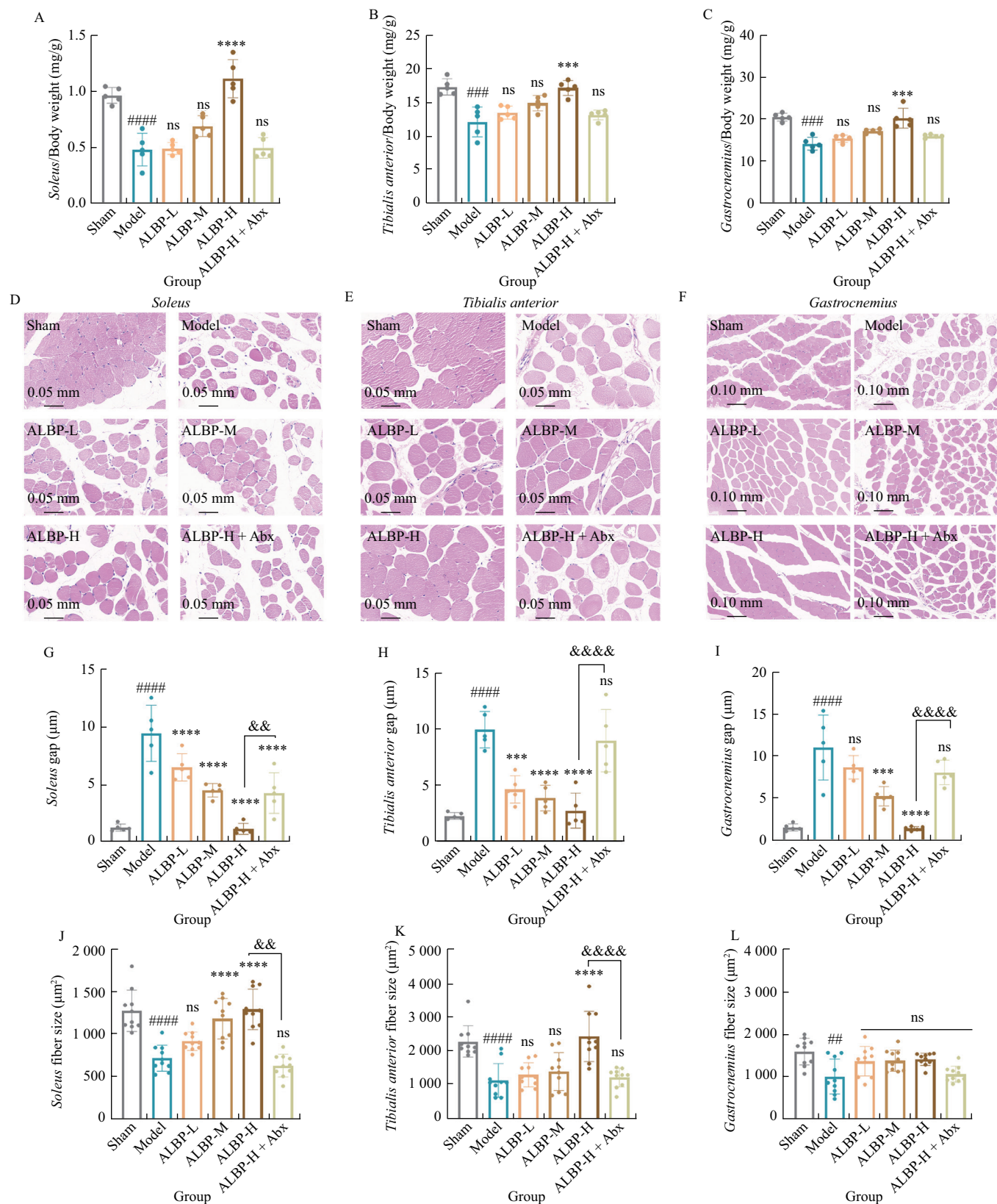


Fig. 8 ALBP displayed dose-dependent manner in improving muscle atrophy by increasing muscle mass. Muscle index of (A) soleus, (B) tibialis anterior and (C) gastrocnemius muscles ($n = 5$). Histological examination of (D) soleus, (E) tibialis anterior and (F) gastrocnemius muscles. Muscle fiber gap analysis of (G) soleus, (H) tibialis anterior and (I) gastrocnemius muscles ($n = 5$). Muscle fiber area analysis of (J) soleus, (K) tibialis anterior and (L) gastrocnemius muscles ($n = 10$).
 && $P < 0.01$ represent ALBP vs. ALBP + Abx groups. The same as below.

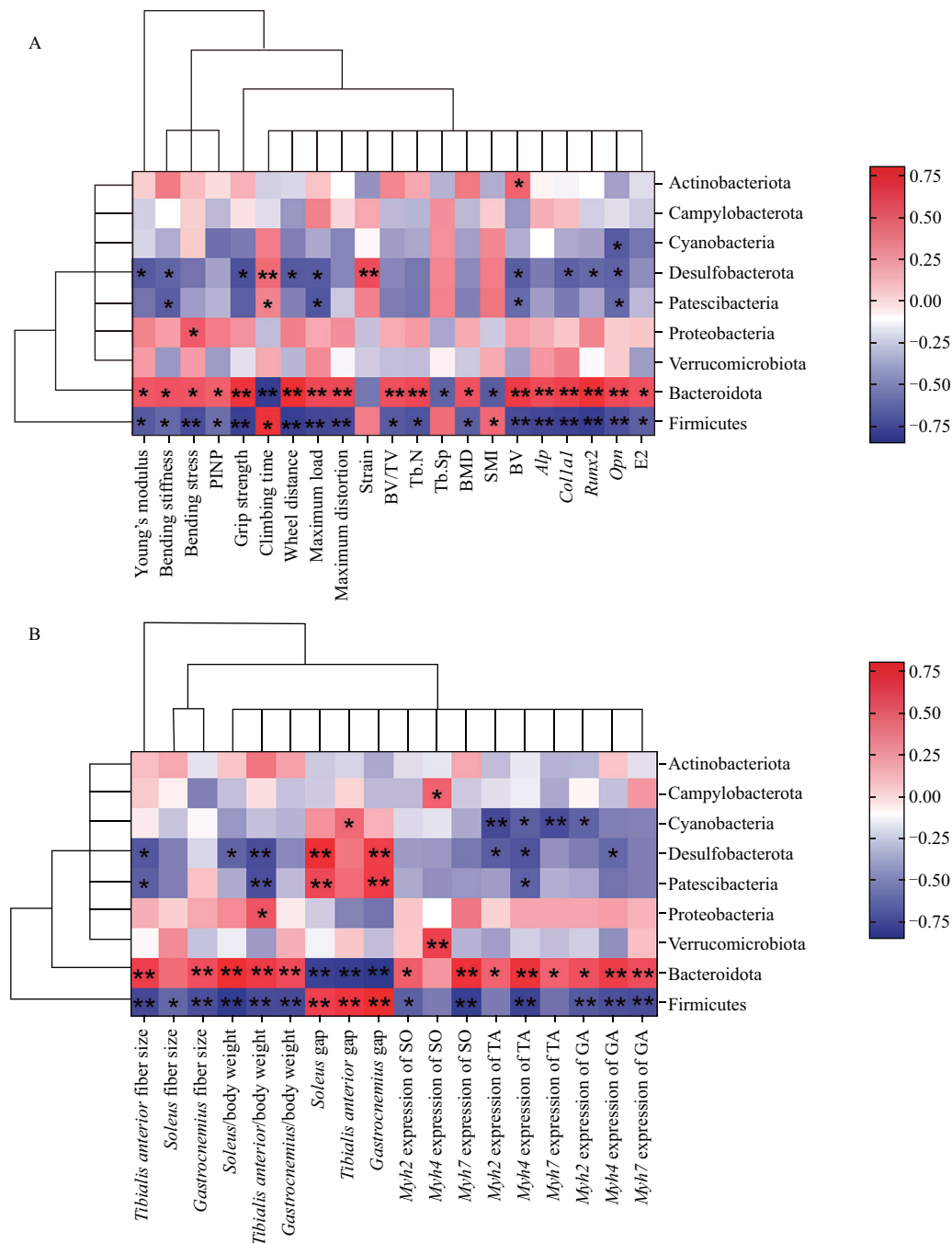


Fig. 9 Correlation between intestinal bacteria and osteosarcopenia. (A) The relationship between intestinal bacteria and osteoporosis. (B) The relationship between intestinal bacteria and sarcopenia.

producing bacteria such as *Bacteroides* and *Alloprevotella*, thus playing an immunomodulatory role^[37]; similarly, *Tremella* polysaccharide has been reported to significantly improve intestinal microbiota diversity, restore the relative abundance of beneficial genera such as *Lactobacillus*, *Bacillus odoros*, *Helicobacter pylori*, Ruminococcaceae, and Marinifilaceae, stimulate Foxp3⁺ T cells, and promote the production of anti-inflammatory cytokines^[38]. Furthermore, emerging evidence suggests a notable association between osteosarcopenia and gut microbiota composition. On the

one hand, research has indicated that bone homeostasis is intricately linked to the gut microbial composition^[39]. Individuals with low BMD exhibit a reduced Firmicutes/Bacteroidota ratio compared to healthy individuals, with lower abundances of *Bifidobacterium* and Lachnospiraceae in osteoporotic patients^[39-40]. For example, wheat germ has been shown to support bone homeostasis by promoting SCFA-producing microbiota^[41]. On the other hand, certain studies suggest that metabolites derived from the gut microbiota may serve as potential biomarkers for sarcopenia in the elderly^[42]. Notably, disruptions in both

the microbiome and metabolome were observed in sarcopenic patients, with significant reductions in bacterial populations such as *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, and *Bifidobacterium adolescentis*, alongside decreases in metabolites like shikimic acid.

Moreover, similar to findings of Li et al.^[34] from *in vitro* simulations, which showed that LBP possess prebiotic properties by modulating gut microbiota metabolites—SCFAs, we also observed that ALBP treatment significantly altered the gut microbiota composition in mice. This treatment regulated the abundance of various gut bacterial types, with a notable increase in the abundance of Lachnospiraceae_NK4A136_group, a gut bacterium known for SCFA production (Fig. 6).

While most studies on LBP have primarily focused on their effects on osteoporosis, the regulation of skeletal muscle has been less explored. To further study the essential role of LBP on osteosarcopenia, we developed a sarcopenia model and investigated the dual effects of LBP on both bone and muscle. We found that ALBP not only improved osteoporosis but also alleviated muscle loss and atrophy (Fig. 4). Li et al.^[43] demonstrated through *in vitro* C2C12 cell experiments that *Polygonatum sibiricum* polysaccharide (PSP) could improve skeletal muscle aging and mitochondrial dysfunction by activating the PI3K/AKT/mTOR signaling pathway, suggesting that PSP holds potential as a therapeutic agent for sarcopenia. Our findings align with these results, as we discovered that ALBP also activates the PI3K/AKT pathway to promote myocyte proliferation and improve muscle wasting (Fig. 5).

Through analysis, this study also found a significant correlation between the composition of intestinal bacteria and osteosarcopenia (Fig. 9), and further proved that ALBP played an important role in improving the intestinal microbes in osteosarcopenia by establishing a pseudo-sterile mouse model (Figs. 7–8).

Despite the promising outcomes, several limitations remain in our study. Further adjustments in dosage and administration frequency are necessary to optimize the therapeutic effects of ALBP. Additionally, single-cell sequencing could be employed to analyze cellular phenotypes and metabolic changes in the mice. Consequently, further investigations utilizing larger experimental models are necessary to fully elucidate the clinical relevance of ALBP.

5. Conclusions

In conclusion, the present study assessed the ameliorative effects of ALBP on osteoporosis and sarcopenia through a comprehensive evaluation of bone and skeletal muscle function in OVX mice model. The findings demonstrate that ALBP significantly enhances bone strength and density, promotes bone formation, reduces bone resorption, increases muscle mass, and alleviates muscle atrophy. Notably, these beneficial effects were closely associated with changes in gut microbiota. Overall, this study suggests that ALBP has a significant nutritional intervention effect on elderly women with osteosarcopenia which shows that it can be used as a functional food to improve osteosarcopenia in postmenopausal women.

Declaration of competing interest

The authors declare no interest conflict. They have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Data availability

The authors declare that all data supporting the results of this study are available within the article and/or from the authors. The sequencing datasets generated for this study have been deposited in the NCBI Sequence Read Archive (SRA) database (accession Nos.: SRP561923, SRP562806, SRP562808).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2025.9250549>.

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