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Gut microbiota dysbiosis and bone mineral density in hemodialysis patients: The mediating role of immune-metabolic pathways and clinical implications for nursing care

Acta Microbiologica et Immunologica Hungarica

73 (2026) 1, 20–32

DOI:
10.1556/030.2026.02826

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Received: November 14, 2025 • Accepted: January 8, 2026
Published online: February 2, 2026

RESEARCH ARTICLE

ABSTRACT

The relationship between gut microbiota dysbiosis and bone mineral density (BMD) in hemodialysis patients, mediated through immune-metabolic pathways, remains to be fully elucidated. In this single-center prospective cross-sectional study, 165 maintenance hemodialysis patients were included to evaluate the independent association between gut microbiota composition and BMD, quantify the mediating roles of immune markers and gut-derived metabolites, and assess the effect modification by nursing-modifiable factors. Fecal samples underwent 16S rRNA sequencing and functional prediction. Inflammatory cytokines (IL-6, TNF-α), gut-derived metabolites (indoxyl sulfate, butyrate), and BMD via dual-energy X-ray absorptiometry (DXA) were measured. Gut microbiota community structure significantly differed across BMD tertiles ($R^2 = 0.033$, $P = 0.003$). After full adjustment, principal coordinate 1 (PCoA-PC1, beta-diversity) was negatively associated with femoral neck BMD, while the Shannon diversity index showed a positive association (both $P < 0.05$). We identified 15 differentially abundant genera between high and low BMD groups. Functional prediction revealed short-chain fatty acid pathways were positively associated with BMD, while indole/p-cresol pathways showed negative associations. Mediation analysis demonstrated that immune markers and gut-derived metabolites collectively explained 45.71% of the microbiota-BMD relationship. Nursing factors significantly modified this association, with the negative relationship strengthened by low fiber intake, severe constipation, proton pump inhibitor use, and inadequate dialysis (Kt/V < 1.4). In conclusion, gut microbiota dysbiosis is independently associated with lower BMD in hemodialysis patients, partially mediated through immune-inflammatory pathways and gut-derived metabolites. Dietary fiber optimization, constipation management, prudent proton pump inhibitor prescribing, and dialysis adequacy represent actionable nursing targets to mitigate gut-mediated bone loss in this vulnerable population.

KEYWORDS

hemodialysis, gut microbiota, bone mineral density, microbiome, immune mediation, nursing intervention

1. INTRODUCTION

After chronic kidney disease enters the dialysis stage, bone mineral density declines and fracture risk increases markedly [1], and although traditional factors such as mineral metabolism abnormalities, age, and body composition are important, they are insufficient to fully explain skeletal phenotypic differences in the dialysis population [2, 3]. Dysbiosis of the gut microbiota is prevalent in the uremic milieu, and its metabolites together with the host immune-inflammatory state can jointly affect bone remodeling [4]. Short-chain fatty acids are considered to have barrier and immunoregulatory effects, whereas indole- and p-cresol-related uremic toxins are considered to impair osteogenesis and enhance inflammation [5].

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In patients on dialysis, dietary fiber restriction, a heavy constipation burden, long-term exposure to proton pump inhibitors, and differences in dialysis adequacy may shape the microbiota and metabolic pathways and thereby alter bone metabolic risk; this microbiota-immune-bone axis from a nursing perspective warrants systematic testing [6, 7]. Existing studies are mostly based on small samples or mixed populations and often describe community composition or individual metabolites at a single dimension [8], lacking a framework that simultaneously integrates community structure, functional pathways, immune inflammation, and bone metabolism indices in dialysis patients. Key confounders such as dialysis vintage, Kt/V, vitamin D, and calcium-phosphorus balance are insufficiently controlled, and unsynchronized sampling and heterogeneous quality control make causal chains difficult to disentangle [9].

Nursing modifiable factors are mostly treated as covariates, with a lack of tests for effect modification of their context dependence, and a lack of quantitative mediation analyses to parse the contribution proportions of immunity and gut-derived metabolism to the link between the microbiota and bone [10]. Establishing actionable intervention targets and risk stratification criteria around the dialysis scenario remains a key gap for clinical translation. Based on a single-center prospective cross-sectional design, this study synchronously obtained, within a unified time window, 16S rRNA sequencing and functional prediction, inflammatory cytokines and gut-derived uremic toxins, dual-energy X-ray absorptiometry (DXA) and bone turnover markers, tested independent associations using prespecified multivariable models, and used parallel mediation and interaction analyses to quantitatively evaluate immune-metabolic pathways and the effect modification by nursing factors. It aimed to elucidate the independent link between dysbiosis and decreased bone mineral density, quantify the contributions of immune and metabolic pathways, and identify translatable targets such as dietary fiber, constipation management, proton pump inhibitor (PPI) prescribing, and dialysis adequacy. The results overview indicates consistency of evidence at the community and functional levels with partial mediation present, and nursing-related contexts can amplify or attenuate adverse associations, pointing to feasible pathways for stratified interventions and efficacy monitoring.

2. MATERIALS AND METHODS

2.1. Study design

This study was a prospective cross-sectional observational study conducted at the hemodialysis center of the Nephrology Department, The Second Medical Center of Chinese PLA General Hospital, Beijing, China, from January 1, 2024 to June 30, 2025, implemented after approval by the institutional ethics committee (approval number H2023110188), with all participants providing written informed consent before enrollment.

2.2. Study participants and sample size estimation

Inclusion criteria were: age 18–80 years; maintenance hemodialysis duration ≥3 months; three dialysis sessions per week, 4 h per session, with a stable dialysis prescription for ≥1 month; and ability to complete dietary records, questionnaire completion, and biospecimen collection. Exclusion criteria were: acute infection within the past 4 weeks; systemic use of antibiotics within the past 4 weeks; a history of inflammatory bowel disease, short bowel syndrome, or extensive intestinal surgery; active malignancy; decompensated liver cirrhosis; pregnancy or lactation; and receipt of broad-spectrum immunosuppressive therapy within the past 4 weeks.

Sample size was estimated based on testing the "independent association between gut microbiota structure indices and bone mineral density (BMD)" as the primary effect. According to an expected correlation strength $r = 0.25$, two-sided $\alpha = 0.05$, and statistical power $(1-\beta) = 0.80$, the sample size required by the correlation test formula was 124. Considering the number of confounders planned to be included in multivariable regression and about 15% of samples being ineligible or having missing data, and further adding about 10% to cover model complexity for interaction and mediation analyses, the final target sample size was set at no fewer than 160, with enrollment completed by consecutive inclusion.

2.3. Data collection procedures

All data were collected within a single observation window to ensure temporal alignment. Each participant completed within the same week: one fasting venous blood draw before a midweek dialysis session; collection of a home fecal sample on the first non-dialysis day of that week, preserved in a room-temperature stabilizing medium and delivered for testing; DXA bone mineral density measurement within ±14 days; and within the same week 3-day dietary records (including 1 dialysis day and 2 non-dialysis days), a physical activity questionnaire, a constipation scale, and medication reconciliation [11]. Nurses recorded the dialysis prescription and adequacy indices in a fixed template and simultaneously cross-checked the medical record entries for the past three months to ensure information consistency. Sample transport used a unified cold chain or stabilizer system, and aliquoting and storage were completed within 2 h after arrival at the laboratory.

2.4. Nursing-related exposures and assessment of dialysis parameters

Dietary intake was assessed using a 3-day weighed food record, completed by participants under nurse guidance; nurses verified items one by one at the next-day follow-up and entered them into nutrition analysis software, and daily dietary fiber intake (g day^{-1}) and energy intake were calculated based on the Chinese Food Composition Table. Constipation burden was scored using the Cleveland Clinic Constipation Scoring System (Chinese version), and weekly

bowel movement frequency and whether laxatives were used were recorded [12]. Medication exposure was verified item by item by nurses according to prescriptions and medication records over the previous three months, with a focus on recording the use status and start and stop times of PPI, phosphate binders, vitamin D preparations, calcium supplements, and prebiotics or probiotics. Dialysis adequacy was calculated using single-pool Kt/V (Daugirdas formula), and dialysis vintage, dry weight, transmembrane pressure, dialyzer model, and whether the dialysate composition was adjusted were recorded. Physical activity was assessed using the IPAQ short form (Chinese version) and converted to metabolic equivalent-minutes.

2.5. Fecal sample collection and microbiome analysis

Participants used a fecal collection tube containing a DNA stabilizer (OMNIgene•GUT OMR-200) to collect the first morning stool at home, thoroughly mixed it according to the manual, stored it at room temperature, and returned it within 48 h. After entry into storage, samples were kept at −80 °C. DNA extraction used the PowerSoil Pro kit with 0.1 mm zirconia bead-beating lysis; negative extraction controls and strain standard positive controls were set in each batch. The 16S rRNA V3–V4 region amplification primers were 341F/806R; after PCR, products were purified and library-prepared, and 2 × 300 bp paired-end sequencing was performed on the Illumina MiSeq platform, with a target of no fewer than 30,000 effective reads per sample [13]. Sequence processing was completed in QIIME2 (version 2023.2), using the DADA2 algorithm for denoising and chimera removal, with taxonomic annotation according to the SILVA 138.1 database; potential contaminant sequences were identified and removed using the decontam package based on negative controls. After rarefaction to a uniform sequencing depth, the Shannon index and Faith's PD index were calculated; β diversity was calculated in parallel using Bray–Curtis and weighted UniFrac distances, principal coordinates analysis (PCoA) was performed, and PC1 was exported as an overall microbiota structure index. Differential abundance analysis used ANCOM-BC2 (version 2.2) at the phylum and genus hierarchical levels [14]. Functional prediction used PICRUSt2 (NSTI threshold ≤2.0) to output pathway abundances related to short-chain fatty acid synthesis and aromatic amino acid metabolism.

2.6. Inflammation/immunity indices and gut-derived metabolite measurements

On a midweek dialysis day, 5 mL of fasting venous blood was collected before dialysis to separate serum and plasma, centrifuged within 30 min, and stored at −80 °C. High-sensitivity C-reactive protein (hs-CRP) was measured by an immunoturbidimetric assay. IL-6, TNF-α, RANKL, and OPG were tested in batches using commercially available enzyme-linked immunosorbent assay kits registered with the national drug regulatory authority, with standard curves and quality-control curves established according to the kit instructions, and three levels (high/medium/low) of internal quality-control materials set in each batch. Fecal short-chain fatty acids (acetate, propionate, butyrate) were quantified by gas chromatography-mass spectrometry; pretreatment used a uniform weighed aliquot, internal-standard calibration, and results were expressed as μmol g^{-1} feces. Plasma indoxyl sulfate and p-cresyl sulfate were quantified by liquid chromatography-tandem mass spectrometry, using stable isotope internal standards, with a linear correlation coefficient $r \geq 0.995$ and a lower limit of quantification no higher than 0.05 μmol L^{-1} [15]. All assays were performed in the same accredited laboratory, and analysts were blinded to group information.

2.7. Measurement of bone mineral density and bone metabolism outcomes

Bone mineral density was measured by DXA on the same device, with measurement sites including L1–L4 of the lumbar spine and the femoral neck, and outputs of BMD (g cm^{-2}), *T*-score, and *Z*-score [16]. The device underwent daily drift calibration using the official phantom, longitudinal stability assessment across months, and technicians received uniform training and passed competency assessment. Bone turnover markers were measured by electrochemiluminescence immunoassay for serum procollagen type I N-terminal propertied (P1NP) and β-C-terminal telopeptide of type I collagen (β-CTX). Calcium, phosphorus, albumin, 25-hydroxyvitamin D, and parathyroid hormone were measured using standard methods in the central laboratory. DXA and laboratory testing were completed within a ±14-day window of sample and questionnaire collection.

2.8. Definitions of covariates, confounding control, and quality management

Prespecified confounders included age, sex, body mass index, diabetes status, dialysis vintage, single-pool Kt/V, 25-hydroxyvitamin D, parathyroid hormone, serum corrected calcium, serum phosphorus, use of vitamin D preparations and calcium supplements, and physical activity level. Nursing exposure variables included daily dietary fiber intake, constipation scale score, use of laxatives, use of PPI, and use status of prebiotics or probiotics within the past three months. All variables had a data dictionary and valid ranges predefined in the database, with double independent data entry and cross-checking. A random 10% sample of participants underwent repeated questionnaire verification and repeat laboratory testing to assess consistency. Timestamps were set for the collection, transport, and storage of fecal and blood samples, with cold-chain temperature and processing time limits recorded. If the missing proportion of a single variable was <10%, multiple imputation was used; if key outcomes or major exposures were missing, no imputation was performed and the cases were excluded from the analyses.

2.9. Statistical analysis

Statistical analysis was completed in R (version 4.3 and above). Normality of continuous variables was determined

by the Shapiro–Wilk test and density plots, with log or Box–Cox transformation applied when necessary; categorical variables were expressed as counts and percentages. Relative abundances of the microbiome, after sum constraint processing, were transformed by the centered log-ratio transformation for regression analysis. Overall population characteristics were compared across BMD tertiles using a linear trend test or the Cochran–Armitage trend test. All hypothesis tests were two-sided with a significance threshold of $\alpha = 0.05$; multiple comparisons for taxa and pathways controlled the false discovery rate by the Benjamini–Hochberg method ($q < 0.10$).

2.9.1. Primary effect: independent association between dysbiosis and bone mineral density. The primary analysis used femoral neck BMD as the dependent variable and PCoA-PC1 score and the Shannon index as the main independent variables to build multivariable linear regression models. Model 1 adjusted for age, sex, body mass index, and diabetes status; Model 2 added dialysis vintage, single-pool Kt/V, 25-hydroxyvitamin D, parathyroid hormone, corrected calcium, and serum phosphorus on the basis of Model 1; Model 3 further added dietary fiber intake, constipation scale score, use of PPI, use of prebiotics or probiotics, and physical activity level on the basis of Model 2. The analysis was repeated with lumbar spine L1–L4 BMD as the dependent variable for validation. Variance inflation factor was used to monitor multicollinearity, and Cook's distance was used to identify high-influence observations, which were examined for robustness in sensitivity analyses. Because the use of vitamin D preparations and calcium supplements are treatment pathway variables (prescriptions driven by prior CKD-MBD severity), including them in the main models could lead to overadjustment or collider bias; therefore, they were not adjusted for in Models 1–3.

2.9.2. Mediation effect: partial mediation by inflammation/immunity and gut-derived metabolites. A causal mediation analysis framework was used to evaluate the total effect, direct effect, and indirect effect between PCoA-PC1 score and BMD. IL-6 and indoxyl sulfate were prespecified as the primary mediators, and the RANKL/OPG ratio and butyrate concentration entered the model as secondary mediators in parallel paths. Mediation analysis was conducted on the covariate set of Model 2, using a nonparametric bootstrap with 5,000 resamples to calculate indirect effects and their 95% confidence intervals; when moderation and mediation coexisted, stratified mediation estimation was used.

2.9.3. Effect modification: interactions and stratification of nursing-modifiable factors. We tested the effect modification of dietary fiber intake, constipation scale score, use of PPI, and single-pool Kt/V on the relationship "PCoA-PC1 score→BMD" by adding interaction terms separately in Model 3 and reporting the regression coefficients of the interaction terms and 95% confidence intervals. For variables with significant interactions, stratification was performed according to prespecified thresholds (dietary fiber intake stratified by sex-specific medians; constipation scale stratified as mild–moderate–severe; Kt/V stratified at 1.4), and trend plots were drawn.

2.9.4. Sensitivity analyses and multiple-comparison correction. Three sensitivity analyses were conducted to verify robustness: (1) repeating all models after excluding participants with infection or antibiotic use within the past 4 weeks; (2) repeating analyses using the first principal coordinate of PCoA based on weighted UniFrac distance instead of Bray–Curtis; and (3) using robust regression instead of ordinary least squares regression. P values for taxonomic stratification differences and functional pathway analyses were adjusted by the Benjamini–Hochberg method and the adjusted q values were reported; core conclusions were judged at $q < 0.10$.

3. RESULTS

3.1. Participant enrollment, baseline characteristics, and quality control

A total of 181 patients were enrolled; the final primary analysis sample size was 165, and the numbers of samples for microbiome, inflammation/immunity, and metabolite analyses were 178, 173, and 169, respectively (Fig. 1). In unadjusted trend tests across BMD tertiles (linear regression for continuous variables; Cochran–Armitage for categorical variables), the low-BMD group showed older age, a lower proportion of males, longer dialysis vintage, a higher proportion of diabetes, lower body mass index, lower single-pool Kt/V, lower daily dietary fiber intake, higher constipation scores, less physical activity, more common use of laxatives and PPI, more common use of phosphate binders, and less use of prebiotics/probiotics (all $p\_trend < 0.05$) (Table 1). Quality-control data showed that effective reads, chimera proportion, taxonomic classification accuracy, functional prediction, metabolite assays, and DXA measurements all met prespecified quality standards, with passing rates exceeding 98% (Table 2).

3.2. Overall characteristics of the intestinal microbiota and associations with nursing exposures

Using PERMANOVA based on Bray–Curtis distance to compare gut community structure across BMD tertiles, there were statistically significant differences among the three groups ($R^2 = 0.033$, $P = 0.003$) with a small effect size; PCoA showed partial overlap of point clouds among groups, with centroids tending to separate (Fig. 2A). Partial Spearman correlations with FDR correction were used. After controlling for confounders, dietary fiber was positively correlated with α diversity and SCFA pathways, and constipation score was positively correlated with PCoA-PC1 and indole/p-cresol pathways. Kt/V and physical activity were respectively positively correlated with Shannon and SCFA, and negatively

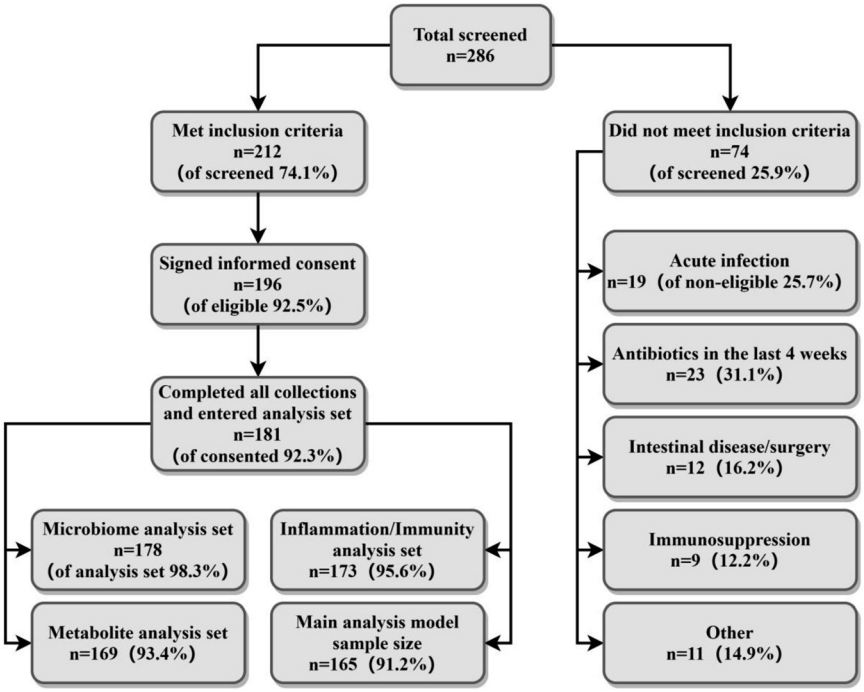

Fig. 1. Flowchart of participant enrollment and analysis populations

Table 1. Baseline characteristics and nursing-related exposures of patients ($\bar{x} \pm s$)/(n, %)

Variable	Overall (n = 165)	Q1 (n = 56)	Q2 (n = 55)	Q3 (n = 54)	p_trend
Age (years)	60.81 ± 11.86	63.71 ± 11.36	60.43 ± 12.02	58.18 ± 11.74	0.011
Sex: male	93 (56.36%)	26 (46.43%)	33 (60.00%)	34 (62.96%)	0.008
Body mass index (kg m^{-2})	22.90 ± 3.12	22.31 ± 3.28	22.87 ± 3.09	23.54 ± 2.92	0.019
Diabetes, n (%)	61 (36.97%)	26 (46.43%)	19 (34.55%)	16 (29.63%)	0.024
Dialysis vintage (years)	4.91 ± 3.13	5.52 ± 3.41	4.87 ± 3.02	4.33 ± 2.88	0.047
Single-pool Kt/V	1.38 ± 0.20	1.32 ± 0.19	1.39 ± 0.21	1.44 ± 0.20	0.004
Daily dietary fiber intake (g day^{-1})	16.20 ± 4.50	14.62 ± 4.38	16.21 ± 4.12	17.83 ± 4.47	0.001
Cleveland Clinic Constipation Scoring System score (points)	11.04 ± 4.12	12.34 ± 4.25	10.92 ± 4.01	9.81 ± 3.76	0.002
Use of laxatives	90 (54.55%)	37 (66.07%)	28 (50.91%)	25 (46.30%)	0.018
Use of PPIs	53 (32.12%)	22 (39.29%)	17 (30.91%)	14 (25.93%)	0.030
Use of phosphate binders	126 (76.36%)	46 (82.14%)	42 (76.36%)	38 (70.37%)	0.044
Use of vitamin D preparations	97 (58.79%)	37 (66.07%)	31 (56.36%)	29 (53.70%)	0.071
Use of prebiotics or probiotics	46 (27.88%)	11 (19.64%)	15 (27.27%)	20 (37.04%)	0.006
Physical activity (MET-min $week^{-1}$)	1638.60 ± 818.25	1418.27 ± 776.41	1633.15 ± 801.66	1872.64 ± 827.22	0.003
Transmembrane pressure (mmHg)	182.46 ± 28.19	185.96 ± 27.76	182.22 ± 28.12	179.06 ± 28.79	0.089
Adjustment of dialysate composition (yes)	38 (23.03%)	16 (28.57%)	12 (21.82%)	10 (18.52%)	0.137

correlated with PCoA-PC1 and indole/p-cresol pathways; PPI use was positively correlated with PCoA-PC1 and indole/p-cresol pathways (all $q < 0.10$) (Fig. 2B).

3.3. Microecological and functional features associated with bone mineral density

Using ANCOM-BC2 with FDR correction, the top 15 differentially abundant genera ranked by |β_ancom| were all significant (all $q < 0.10$); eight genera were enriched in low BMD (Q1) and seven genera were enriched in high BMD (Q3); β_ancom > 0 indicates higher abundance in Q1 and β_ancom < 0 indicates higher abundance in Q3 (Fig. 3A). Using multivariable linear regression with BH-FDR correction for eight prespecified functional pathways, seven significant pathways were identified (all $q < 0.10$): short-chain fatty acid (SCFA) synthesis pathways were positively associated with femoral neck BMD (3 significant), and indole/p-cresol production pathways were negatively associated (4 significant). $\beta > 0$ indicates that each +1 SD in pathway abundance corresponds to an increase in BMD, and $\beta < 0$ indicates a decrease (Fig. 3B).

Table 2. Summary of experimental and sequencing quality control (reads, coverage, negative/positive controls, and batch assessment)

Item	Overall value	Qualification threshold	Passing rate (%)
Raw reads per sample (reads)	64721.38 ± 12498.64	–	–
Effective reads after denoising (reads)	57983.41 ± 10872.55	≥30,000 reads	98.34
Chimera proportion (%)	2.87 ± 0.96	≤5.00%	99.45
Reads in negative controls (reads)	76 [49–118]	<1% of the minimum sample effective reads	100
Number of ASVs* after decontamination (*n*)	172.47 ± 38.12	–	–
Proportion of samples meeting rarefaction depth threshold (%)	98.34	Effective reads ≥30,000	98.34
Proportion classified to genus level by SILVA (%)	88.92	≥80.00%	100
PICRUSt2 NSTI	0.13 ± 0.05	≤2.00	100
GC-MS calibration curve r for short-chain fatty acids	Acetate 0.9992; propionate 0.9990; butyrate 0.9987	≥0.9950	100
LC-MS/MS method limits of detection for uremic toxins (μmol L^{-1})	Indoxyl sulfate 0.04; p-cresyl sulfate 0.05	≤0.05	100
Within-/between-batch coefficient of variation (%)	Within-batch 5.72; between-batch 7.18	≤10.00%	100
DXA phantom drift (%)	0.42	≤1.00%	100

*ASV: Amplicon Sequence Variant.

3.4. Primary effect: independent association between microbiota structure and bone mineral density

Using OLS with a three-step adjustment of covariates. After full adjustment (Model 3), PCoA-PC1 was negatively associated with femoral neck BMD and the Shannon index was positively associated (both $P < 0.05$); the effects remained stable across stepwise adjustments, model fit and diagnostics were good, and there were no obvious multicollinearity or high-influence points (Table 3).

3.5. Mediation effect: inflammation/immunity and gut-derived metabolites

Causal mediation analysis (bootstrap 5,000 times). Under covariate control, the total effect of PCoA-PC1 on femoral neck BMD was significant (TE = -0.0140 g cm^{-2} per unit PC1, $P = 0.001$). All four prespecified mediators (IL-6, RANKL/OPG, butyrate, and indoxyl sulfate) had significant indirect effects (each ACME $P < 0.05$). Combined ACME = -0.0064 ($P = 0.002$), with a proportion mediated of 45.71%; the direct effect remained significant (ADE $P = 0.009$), indicating partial mediation (Table 4).

3.6. Effect modification analysis of nursing factors

Interaction terms were tested using OLS (Model 3 fully adjusted). The negative relationship between PCoA-PC1 and femoral neck BMD was strengthened with greater constipation burden and with PPI use (both $P < 0.05$); it was attenuated with higher dietary fiber and with Kt/V ≥ 1.4 (both $P < 0.05$) (Fig. 4A–D).

3.7. Secondary outcomes and robustness verification

Using multivariable linear regression (P1NP and β-CTX were log-transformed and expressed as percentage change). Lumbar spine L1–L4 BMD was positively associated with Shannon and negatively associated with PCoA-PC1; P1NP was positively associated with Shannon and negatively associated with PCoA-PC1; β-CTX was negatively associated with Shannon and positively associated with PCoA-PC1 (all $P < 0.05$) (Fig. 5A). The main models were refit and the percentage change in β was calculated (normal-approximation 95% CI). Under three settings—excluding participants with infection or antibiotic use within the past 4 weeks, using weighted UniFrac-PC1, and using robust regression—the direction of the primary effects was consistent, magnitude changes were small, 95% CIs all crossed 0, no substantive shifts were observed, and estimates were robust (Fig. 5B).

3.8. Data integrity and analysis population

Key exposures and outcomes (PCoA-PC1, Shannon, femoral neck BMD) had no missingness; the missing rates of other variables were all <5%, and imputation was completed. After imputation, the analysis samples of the three models were consistent ($N = 165$), and the integrity of the analysis population was good (Table 5).

4. DISCUSSION

After adequate adjustment for age, sex, body mass index, diabetes, and dialysis-related mineral metabolism and lifestyle, community structure and diversity remained stably associated with femoral neck BMD; although the overall structural differences had a small effect size, the directions were consistent and were externally corroborated by lumbar spine BMD and bone turnover markers. At the genus level, the low-BMD group was enriched for conditionally pathogenic and toxin-producing genera, whereas the high-BMD group was enriched for acid-producing genera related to butyrate and propionate generation, suggesting a layered

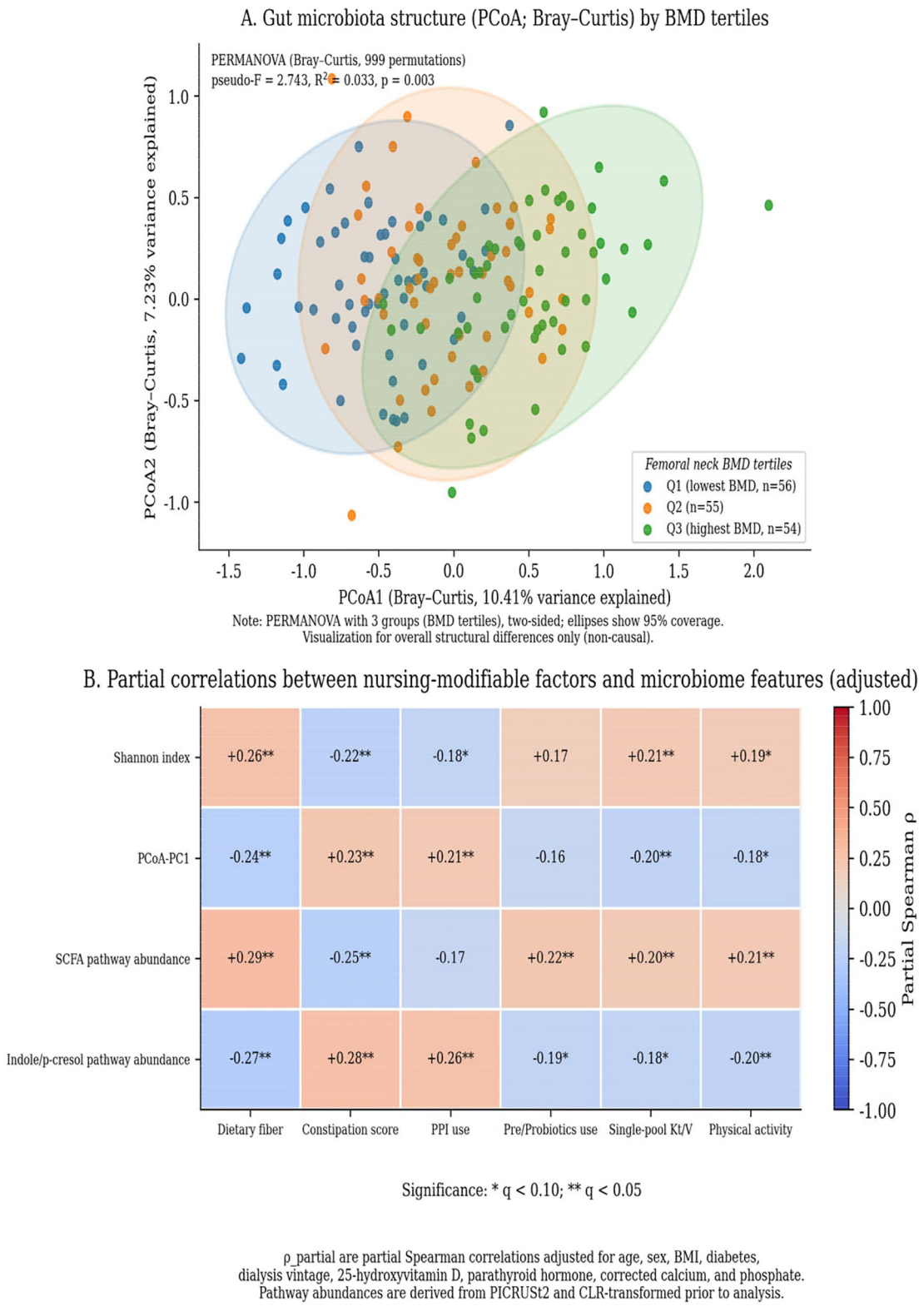

Fig. 2. Analysis of gut microbiota structure and its associations with nursing factors. A: Gut microbiota structure (PCoA; Bray-Curtis) by BMD tertiles. B: Partial correlations between nursing-modifiable factors and microbiome features

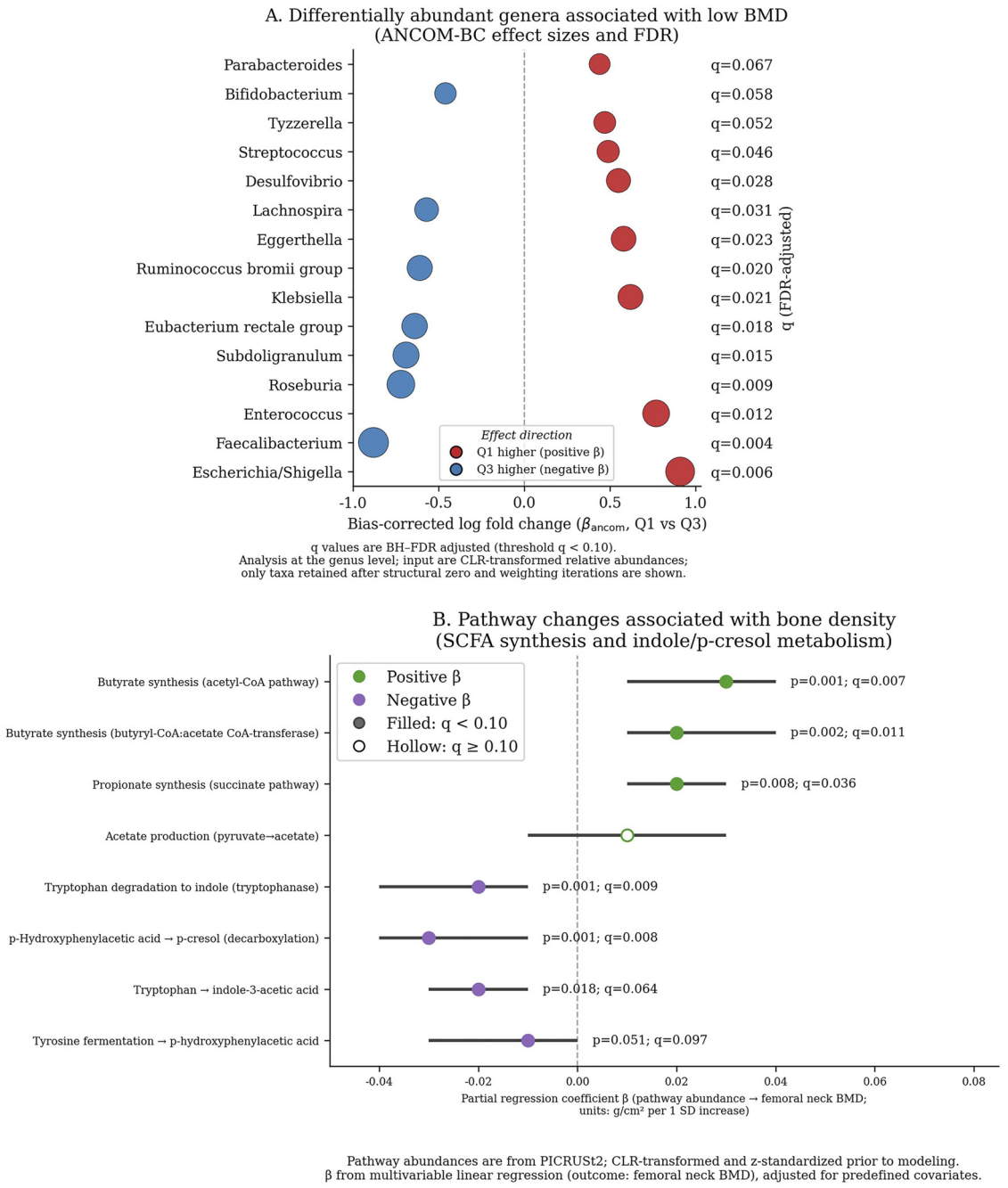

Fig. 3. Differential analysis of microbiota and functional pathways associated with bone mineral density. A: Differentially abundant genera associated with low BMD. B: Pathway changes associated with bone density

convergence from community to genus [17]. This pattern fits the ecological context of dialysis patients dominated by gut-derived inflammation and protein fermentation, in which decreased diversity and metabolic DE functionalization weaken mucosal barrier and bone-protective signals [18]; increased endotoxin and gut-derived uremic toxin burdens promote osteoclast activity via elevated inflammatory cytokines and RANKL/OPG imbalance [19], while a reduction in short-chain fatty acid–producing bacteria leads to insufficient Treg-mediated anti-inflammatory and osteogenic support, and the two together increase the risk of bone mass loss. The independence observed in multivariable models suggests that this link is not explained by traditional confounding, and the consistency in direction between bone resorption and formation markers and community structure in secondary outcomes further strengthens credibility. Prior studies in chronic kidney disease and dialysis populations have widely reported associations of low diversity, enrichment of pro-inflammatory taxa, and reduction of short-chain fatty acid–producing bacteria with abnormalities in bone metabolism [20]. In the hemodialysis setting, this study forms a closed loop of evidence across overall structure,

Table 3. Multivariable regression: gut microbiota indices and femoral neck bone mineral density (Models 1–3)

Independent variable	Model 1 β (95% CI, *P*)	Model 2 β (95% CI, *P*)	Model 3 β (95% CI, *P*)
PCoA-PC1	−0.02 (−0.03, −0.01), 0.001	−0.02 (−0.03, −0.01), 0.002	−0.01 (−0.02, −0.01), 0.003
Shannon index	0.02 (0.01, 0.03), 0.003	0.01 (0.01, 0.02), 0.005	0.01 (0.01, 0.02), 0.010
Model diagnostics (summary)	Maximum VIF 2.31; Cook's distance >4/*n*: 1	Maximum VIF 3.07; Cook's distance >4/*n*: 1	Maximum VIF 3.42; Cook's distance >4/*n*: 2

Note: sample size $N = 165$, model $R^2 = 0.34$, AIC = 112.59. Ordinary least squares regression (OLS). Model 1 adjusted for age, sex, body mass index, and diabetes status; Model 2 added dialysis vintage, Kt/V, 25-hydroxyvitamin D, parathyroid hormone, corrected calcium, and serum phosphorus; Model 3 further added dietary fiber intake, constipation scale score, PPI, prebiotics/probiotics, and physical activity. β indicates the absolute change in femoral neck BMD (g cm^{-2}) per 1-unit increase in the independent variable.

genus distribution, and bone metabolic phenotypes, and, under rigorous quality control and confounding control, provides a translational evidence chain that clarifies verifiable targets and bases for population stratification for subsequent nursing and nutrition intervention studies targeting the microbiota and inflammation pathways.

At the functional level, short-chain fatty acid–related pathways were positively associated with bone mineral density, whereas indole- and p-cresol–producing pathways were negatively associated; parallel mediation analysis of inflammation and gut-derived metabolites suggested that IL-6, RANKL/OPG, butyrate, and indoxyl sulfate together explained nearly half of the link between the microbiota and bone mineral density, with a remaining portion borne by parallel pathways not captured by the included indicators. This pattern is consistent with the synergy between gut metabolism and bone immunity, whereby short-chain fatty acids enhance the epithelial barrier, promote regulatory T-cell (Treg) polarization, and maintain bone homeostasis by suppressing inflammatory cytokine release and reducing osteoclast signaling [21]; elevated IL-6 and RANKL/OPG imbalance drive osteoclast differentiation and activation, and insufficient butyrate weakens this inhibitory effect [22]; indole uremic toxins, through oxidative stress and bone cell signaling, suppress osteogenesis, promote inflammation, and shift bone turnover toward the resorptive side [23]. The proportion mediated is not the entirety, suggesting that dialysis-related mineral and bone disorder, nutritional intake, and body composition still affect bone mass via independent pathways, and interpretation of the causal chain should remain cautious [24]. Consistent with human and animal evidence in chronic kidney disease and dialysis populations that "short-chain fatty acids favor bone health, whereas indole- and p-cresol–related toxins are detrimental to bone mass" [25], this study used quantitative mediation to

Table 4. Mediation effects of inflammation and gut-derived metabolites in the impact of microbiota structure on bone mineral density

Mediator path	ACME (estimate, 95% CI, *P*) (g cm^{-2})	ADE (estimate, 95% CI, *P*) (g cm^{-2})	Total effect (estimate, 95% CI, *P*) (g cm^{-2})	Proportion mediated (%)
IL-6	−0.0022 (−0.0039, −0.0010), 0.004	−0.0118 (−0.0191, −0.0057), 0.001	−0.0140 (−0.0223, −0.0072), 0.001	15.71
RANKL/OPG	−0.0016 (−0.0031, −0.0004), 0.016	−0.0124 (−0.0202, −0.0061), 0.001	−0.0140 (−0.0222, −0.0073), 0.001	11.43
Butyrate	−0.0011 (−0.0021, −0.0002), 0.030	−0.0129 (−0.0204, −0.0066), 0.001	−0.0140 (−0.0220, −0.0071), 0.001	7.86
Indoxyl sulfate	−0.0015 (−0.0029, −0.0005), 0.012	−0.0125 (−0.0200, −0.0062), 0.001	−0.0140 (−0.0221, −0.0070), 0.001	10.71
Combined mediation (parallel)	−0.0064 (−0.0095, −0.0034), 0.002	−0.0076 (−0.0143, −0.0019), 0.009	−0.0140 (−0.0221, −0.0072), 0.001	45.71

Note: causal mediation analysis used PCoA-PC1 as the exposure and femoral neck BMD as the outcome, conducted under the covariate set of Model 2 (age, sex, body mass index, dialysis vintage, Kt/V, 25-hydroxyvitamin D, parathyroid hormone, corrected calcium, and serum phosphorus); ACME denotes average causal mediation effect, ADE denotes average direct effect.

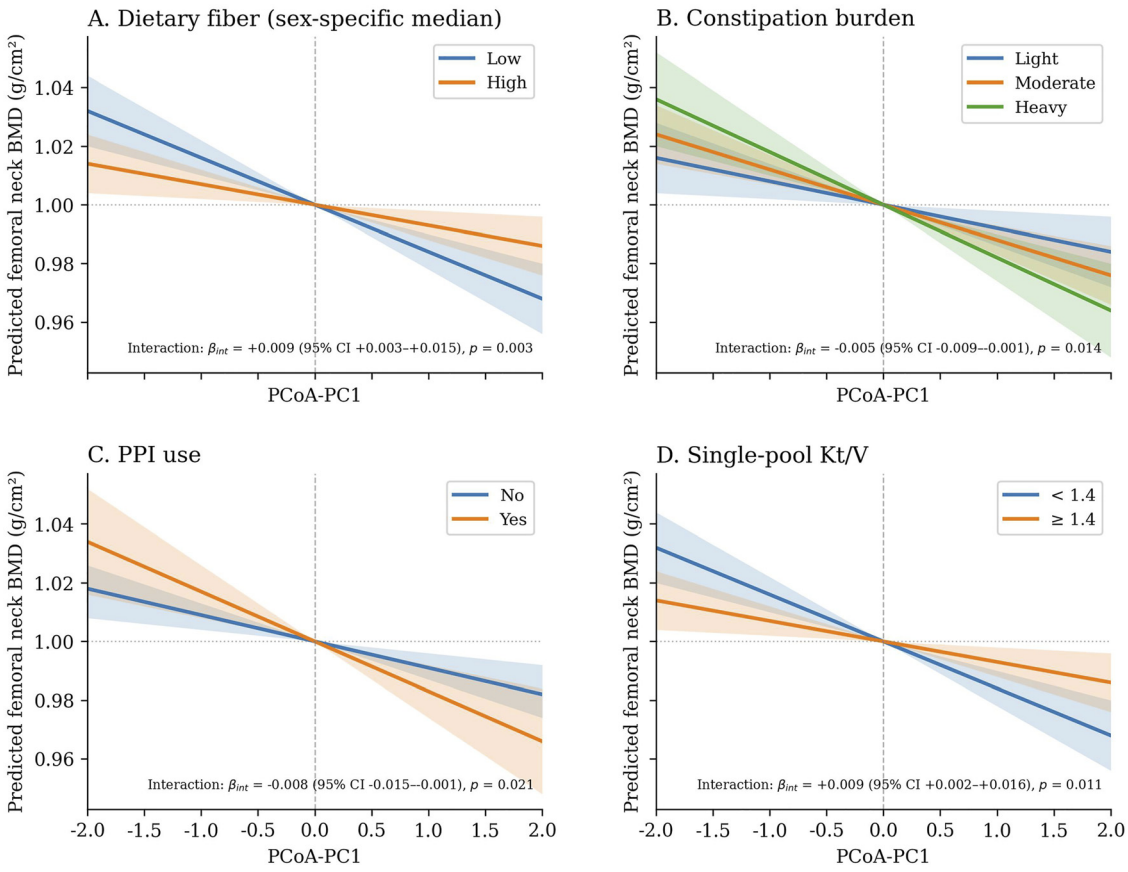

Fig. 4. Effect modification of nursing factors on the relationship between microbiota structure and bone mineral density. A: Dietary fiber. B: Constipation burden. C: PPI use. D: Single-pool Kt/V

parse the contributions of immunity and metabolism, and results consistent in direction with bone turnover markers mutually support each other; after controlling for major confounders, the findings remained robust, indicating that changes in microbiota function are not merely accompanying phenomena but have biological significance, providing a basis for using functional pathways as markers for risk stratification and efficacy monitoring.

Nursing modifiable factors showed systematic correspondences with the microbiota and its functional pathways: dietary fiber and dialysis adequacy were linked to higher diversity and short-chain fatty acid–related pathways, whereas constipation burden and use of PPI were linked to DE functionalization of the community and indole/p-cresol pathways; in interaction analyses, these factors altered the slope of the relationship between microbiota structure and femoral neck BMD, suggesting that under low fiber, severe constipation, PPI use, or inadequate dialysis, bone mass is more susceptible to adverse microbiota influences, whereas under high fiber intake and adequate dialysis, this adverse association was attenuated [26]. Biologically, soluble and insoluble fiber, via increased production of short-chain fatty acids, strengthen the epithelial barrier and immune homeostasis, reducing inflammatory and osteoclast signaling [27]; delayed defecation increases protein putrefactive fermentation and endotoxin exposure and suppresses colonization of acid-producing taxa [28]; gastric acid suppression is associated with proximal small intestinal bacterial overgrowth and may increase pro-inflammatory metabolic flux [29]; adequate dialysis can reduce the body burden of gut-derived uremic toxins and mitigate systemic inflammation. Prior studies have reported that high-fiber diets and prebiotic interventions improve diversity and short-chain fatty acid levels, that constipation and exposure to PPI are associated with dysbiosis, and that dialysis adequacy is associated with reduced uremic toxins [30]; under uniform adjustment and robustness checks, this study identified susceptible subgroups and protective contexts in the form of effect modification, suggesting that nursing strategies centered on dietary prescription, constipation pathway management, PPI prescribing, and improving dialysis adherence have priority translational value and can be used to formulate stratified follow-up and intervention intensity.

The study has several limitations. The single-center, prospective cross-sectional design limits interpretation of associations due to uncertain temporality and center-specificity; 16S sequencing and PICRUSt2 are functional prediction and cannot replace direct quantification by metagenomics and metabolomics; even under extensive confounding control, residual factors may remain, including

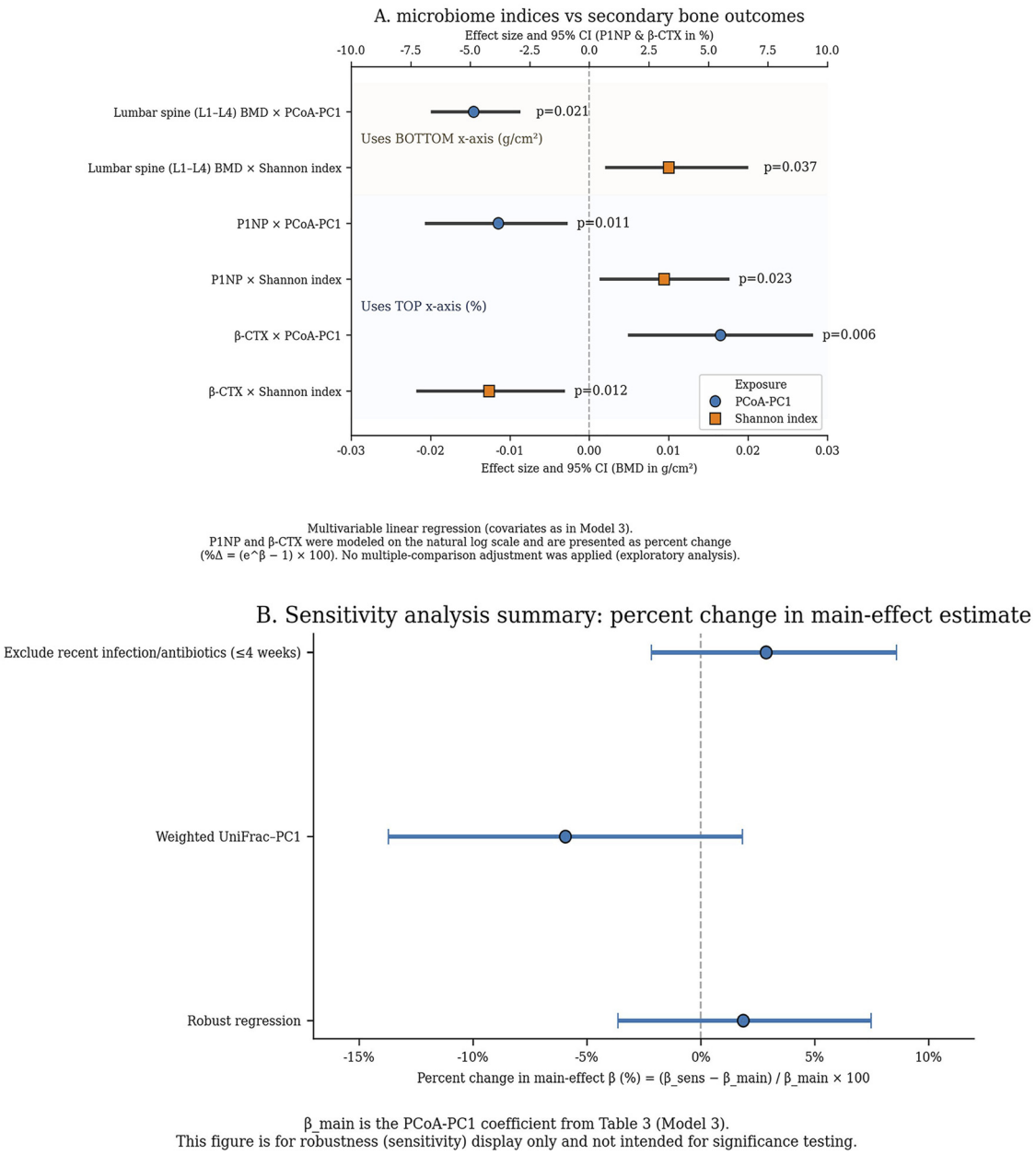

Fig. 5. Effects of the microbiome on secondary bone outcomes and sensitivity analyses. A: microbiome indices vs secondary bone outcomes. B: sensitivity analysis summary

dietary micronutrients, the spectrum of inflammatory comorbidities, and medication dosage and duration; the multiple-comparison threshold used was $q < 0.10$ and effect sizes were overall small, requiring replication in larger samples; fecal and blood samples were at a single time point and are limited in capturing diurnal and weekly fluctuations of microbial metabolism and inflammation. Future studies should conduct multicenter longitudinal follow-up with synchronized repeated DXA and concurrent fecal and blood sampling to establish clear temporality; pragmatic randomized trials in routine nursing pathways should evaluate the individual and combined effects of high-fiber dietary prescriptions and nutritional follow-up, comprehensive constipation management, dose reduction or substitution of PPI, prebiotic or probiotic regimens, and optimization of dialysis adequacy; use metagenomics combined with targeted metabolomics and multiple inflammatory indicators, together with causal mediation and structural equation modeling, to verify the microbiota-immune-bone axis and quantify the contributions of each pathway; improve measurement of exposures and adherence by including records of micronutrients and protein sources, adherence to bowel-regimen strategies, and medication dosage and duration, and report according to a preregistered protocol with data and code sharing to improve reproducibility and generalizability.

Table 5. Summary of missing data and analysis populations (imputation status and sample sizes of each model)

Variable name	Missing (n)	Missing %	Multiple imputation (yes/no)
PCoA-PC1	0	0	No
Shannon	0	0	No
Femoral neck BMD	0	0	No
Age	0	0	No
Sex	0	0	No
Body mass index	2	1.21	Yes
Diabetes	1	0.61	Yes
Dialysis vintage	1	0.61	Yes
Single-pool Kt/V	1	0.61	Yes
25-hydroxyvitamin D	3	1.82	Yes
Parathyroid hormone (PTH)	2	1.21	Yes
Corrected calcium	1	0.61	Yes
Serum phosphorus	2	1.21	Yes
Daily dietary fiber	6	3.64	Yes
Cleveland Clinic Constipation Scoring System score	5	3.03	Yes
Use of PPI	2	1.21	Yes
Use of prebiotics/probiotics	3	1.82	Yes
Physical activity (MET-min week^{-1})	7	4.24	Yes
IL-6	4	2.42	Yes
RANKL/OPG ratio	5	3.03	Yes
Fecal butyrate	6	3.64	Yes
Plasma indoxyl sulfate	7	4.24	Yes

Note: The denominator is the primary analysis sample $n = 165$; percentage = missing $n/165 \times 100\%$. Variables with missing >0 underwent multiple imputation (MICE: $m = 20$, iterations = 10, PMM $k = 5$).

5. CONCLUSIONS

In the maintenance hemodialysis population, dysbiosis is independently and stably associated with lower bone mineral density, characterized by decreased diversity, enrichment of pro-inflammatory taxa, and reduction of short-chain fatty acid–producing bacteria. At the functional level, short-chain fatty acid synthesis pathways are positively associated with bone mass, and indole/p-cresol metabolic pathways are negatively associated; inflammation and gut-derived uremic toxins mediate only part of the link between the microbiota and bone, suggesting that the microbiota-immune-bone axis operates through multiple pathways. Dietary fiber, constipation burden, use of PPI, and dialysis adequacy can alter the microbiota and its relationship with bone mineral density and have actionable nursing intervention value. A comprehensive strategy centered on dietary prescription, constipation management, optimization of proton pump inhibitor use, and improving Kt/V is expected to reduce the risk of bone mass loss by improving the microbiota and inflammatory status and to be used for risk stratification and efficacy monitoring.

Declaration of interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement: This study was conducted in accordance with the ethical standards of the institutional and national research committees and the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of The Second Medical Center of Chinese PLA General Hospital (Approval No. H2023110188). Informed consent was obtained from all participants prior to inclusion in the study.

Data availability: All data are available on request from corresponding author.

Funding: This research was financially supported by the Nephrology department, The Second Medical Center of Chinese PLA General Hospital, 100039, Beijing, China.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent to publication: Not applicable.

Conflict of interest: Not applicable.

Clinical trial: Not applicable.

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