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Racial and Ethnic Differences in Studies of the Gut Microbiome and Osteoporosis

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Abstract

Purpose of Review The purpose of this review is to summarize the scientific evidence published in the past 5 years examining the epidemiology of bone health as it relates to the gut microbiome, across race and ethnicity groups.

Recent Findings The link between the gut microbiome and bone health is well established and is supported by numerous biological mechanisms. However, human study research in this field is dominated by studies of older adults residing in Asian countries. A limited number of epidemiological and randomized controlled trials have been conducted with individuals in other countries; however, they are marked by their racial and ethnic homogeneity, use varied measures of the gut microbiome, and different interventions (where applicable), making comparisons across race and ethnic groups difficult.

Summary As the global prevalence of osteoporosis increases, the need for lifestyle interventions is critical. Existing data suggest that racial and ethnic differences in gut microbiome exist. Studies examining the relation between bone health and gut microbial structure and function across diverse racial and ethnic groups are needed to determine appropriate microbiome-based interventions.

Keywords Osteoporosis · Gut microbiome · Bone health · Racial and ethnic differences · Epidemiology

Introduction

Osteoporosis (OP) is a metabolic disease of low bone mass paired with weak bone tissue and strength that can lead to life-threatening fragility fractures. OP is a global public health issue that is increasing in prevalence as the population ages, with a current worldwide prevalence in women of 23% and in men of nearly 12% [1]. OP is multifactorial in its etiology, providing numerous potential points of intervention in the development and/or progression of disease. The gut microbiome (GM), an endocrine organ comprising trillions of microorganisms that reside within the human intestines, has been established to play a role in the health of bone [2] and has received increasing attention as a promising modifiable point of intervention [3].

Effective OP interventions require a clear understanding of the physiological mechanisms linking the GM with the prevention and development of OP, in addition to knowledge about how these pathways may vary by age, sex, health status, socioeconomic status, and race. Composition and function of the GM are influenced and vary by race and ethnicity [4]. Recent data from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) revealed a unique gut microbial composition based on migration status and a difference in the GM compared to other race/ethnic groups in the USA [5]. A multiethnic study of adults living in Malaysia showed that ethnicity was most significantly associated with the GM, compared to other factors such as diet, health, demography, and hygiene [6••]. Results from the Healthy Life in an Urban Setting study demonstrated a shared gut microbial composition with those of the same ethnicity, supporting findings from the American Gut Project and the Human Microbiome Project which revealed differences in gut microbial composition between the four represented ethnicities [7, 8]. Finally, a study of East Asian

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and White individuals living in San Francisco, California reported differences between the groups in bacterial richness, community structure, and genetic potential functional pathway enrichment [9•]. These studies demonstrate clear differences in GM composition and function across ethnic and race groups.

As with gut microbial composition, differences exist in OP screening, prevalence of OP, fracture risk and rate, OP care, and subsequent disability and/or mortality. In the USA, Black and Hispanic women are screened less often for OP than non-Hispanic White women despite the fact that Black women have been shown to experience a higher proportion of fragility fractures and higher risk of treatment disparities, post-fracture disability, and mortality than non-Hispanic White women [10]. Studies including Hispanic and Asian subgroups, as well as Native American representation, are lacking, but necessary, based on available data [10, 11]. A 2014 review identified a wide range in the global prevalence of OP within industrialized countries (from 2% in Australia to 26.3% in Japan) [12]. In a recent meta-analysis, the lowest overall prevalence of OP was reported in a Canadian study (1.07%) and the highest in an Iranian study (77.3%), with subgroup analysis by gender showing the highest prevalence in men to be in Asia, and among women, in Africa [1].

Differences in GM composition and function may explain, in part, observed differences in OP prevalence and incidence of fracture across ethnic/race groups. Hypothesized mechanisms by which this relation differs are multifactorial. Genetic variations are an accepted contributor to differences in bone outcomes, and while host genetics has been estimated to account for 1.9–8.1% of the observed differences in GM structure and function, this number requires additional investigation [4, 6]. Different cultural practices related to traditional remedies, breastfeeding, physical activity, and sun exposure could influence the GM-OP relation. Different dietary practices are a likely factor impacting the composition and function of the GM as well as the skeletal system. Finally, differences in the built environment are also a potential contributor and include such factors as living in a rural versus urban setting and exposure to environmental heavy metals.

Observed differences in the GM and the prevalence of OP and related fracture suggest that gut microbial risk factors and interventions to alter the GM to improve bone health may need to be race and/or ethnicity specific; however, observational studies examining the relation of the GM with bone outcomes in diverse populations are lacking. Without epidemiological studies, informed clinical trials cannot occur. This review reports on the state of the research published in the past 5 years describing the GM in relation to bone turnover markers (BTMs), bone mineral density (BMD), and other measures of bone health in observational and randomized controlled studies (summarized in Table 1). Suggested mechanisms underlying the pathway from the GM to bone health are also reviewed.

Methods for studying the GM include marker gene analysis and metagenomic analysis. The difference is sequencing based on targeting an amplicon of only one gene, typically the 16S ribosomal RNA (rRNA) gene in a sample versus sequencing all or most genes in a sample with the goal of determining the comprehensive functional potential of the GM. The 16S rRNA gene sequencing provides information on microbial diversity and composition, allowing for taxonomic classification of sample microbiota, while metagenomic sequencing reports functional pathways of the microbial DNA. Tools such as PICRUSt allow for prediction of the functional potentials of microbial communities based on their taxonomic composition (from 16S rRNA gene sequencing). This review includes studies that report varying methods for studying the GM. Differences in outcomes between studies should consider that differing methods could, in part, explain differences in results.

Epidemiological Studies

The theory suggesting an association between the GM and low BMD was introduced in a 2001 prospective cohort study examining small intestinal bacterial overgrowth and metabolic bone disease [13]. Since that time, epidemiological studies have filled gaps in the literature and informed preclinical studies and randomized controlled trials. A recent meta-analysis of 12 epidemiological studies testing associations between the gut microbiota and OP showed that the preponderance of the latest human data were cross-sectional, conducted among adults living in Asian countries and utilized 16s rRNA gut microbial gene sequencing methodology [14••]. Five observational studies included in the present review, including two not considered in the recent meta-analysis, also conducted metagenomic sequencing [15–19]. Ling and colleagues also reported on imputed functionality based on the composition of taxa determined to be present by16s rRNA gene sequencing [20•]. Women were the focus of all but five of the observational studies in the current review, with variation in their average age, menopausal status, and degree of bone health that ranged from 40 to mid-80s, from pre- to post-menopausal, and from healthy BMD to senile OP [15, 19–22]. Overall, results across these studies are inconsistent. Measures of bacterial richness and diversity among people with healthy versus unhealthy bone vary across studies. In addition, there is no agreement on which gut microbial species, or their function, are consistently related to bone outcomes. The subsections below outline important differences across these recent studies.

Table 1 Associations of gut microbiome with measures of bone health in clinical and epidemiological studies from the past 5 years

Study design	Population, *n*	Exposure or intervention	Outcome measures	Results	References
RCT (24 weeks)	Women Spanish origin Mean age 56, *n* = 79	Enriched dairy product	BMD; BM; BTMs	Enriched product increased bone mass, maintained BMD compared to placebo.	Morato-Martinez et al. (2020); data collected Jan–July 2015
Multicenter RCT (12 months)	Women Swedish origin Mean age 59, *n* = 249	*Lactobacillus* strains (3) supplementation	BMD; BTMs	*Lactobacillus* maintained LS-BMD compared to placebo loss in LS-BMD; no difference in BTMs.	Jansson P.-A., et al. (2019); data collected in 2017
RCT (12 months)	Women Swedish origin Mean age 76, *n* = 90	*Lactobacillus reuteri* supplementation	Tibia total volumetric BMD, bone microarchitecture, BTMs	*L. reuteri* reduced bone loss compared to placebo.	Nilsson A. G., et al. (2018); data collected 2016–2017
RCT (24 weeks)	Women Japanese origin Mean age 58, *n* = 76	*Bacillus subtilis* supplementation	16s rRNA gene seq; BMD; BTMs	*Bacillus subtilis* increased total hip BMD and decreased abundance of genus *Fusobacterium* compared to placebo.	Takimoto T., et al. (2018)
Cross-sectional	Women and men Chinese origin Mean age = 60, *n* = 113	Stool samples; DXA	16s rRNA gene seq (113 ppts); metagenomic seq (57 ppts); BMD	Higher relative abundance of *Firmicutes* and *Clostridiales* and BMD; positive correlation between GM gene numbers and BMD in women.	Wang Y., et al. (2022); data collection ~2018
Cross-sectional	Women Chinese origin Mean age 53, *n* = 499	Stool and blood samples; DXA at femoral neck	Metagenomic seq; serum metabolomics; BMD	*Bacteroidetes* and *Fusobacteria* negatively associated with BMD; *Firmicutes* positively associated; several metabolic pathways in GM associated with BMD.	Greenbaum J, et al. (2022); data collection date not reported
Cross-sectional	Women and men Chinese origin Mean age 64, *n* = 1776	Stool and blood samples; DXA	16s rRNA gene seq; potential pathway analysis; targeted metabolomics; BMD	Differing bacteria positively or negatively associated with OP depending on site, possibly through peptidase and transcription pathways.	Ling C. W., et al. (2021); data collected 2008–2013
Case control	Women Chinese origin Mean age 69, *n* = 108	Stool samples; DXA	16s rRNA gene seq; potential pathway analysis; BMD	*Bacteroidetes*-dominated microbiome positively associated with OP (LS); 10 KEGG pathways enhanced in OP (LS).	Wei M., et al. (2021); data collected 2018–2019
Cross-sectional	Women New Zealand origin Mean age 62.5, *n* = 86	Stool samples; DXA	Shotgun metagenomic seq; BMD	Numerous bacteria positively and negatively associated with OP; several enhanced KEGG pathways in OP compared with healthy.	Rettedal E. A., et al. (2021); data collected 2017–2018

Table 1 (continued)

Study design	Population, *n*	Exposure or intervention	Outcome measures	Results	References
Cross-sectional	Women Chinese origin Mean age 61, $n = 97$	Stool and blood samples; DXA	16s rRNA gene seq; BTMs; BMD	Taxon-specific bacterial differences associated with CTX; no differences in taxa associated with BMD in OP compared to healthy.	Chen L., et al. (2021); data collected 2020
Cross-sectional	Women Chinese origin Mean age 61, $n = 42$	Stool samples; DXA	16s rRNA gene seq; BMD	Decreased proportion of *Prevotella* in OP (FN and LS) compared to osteopenia (~60%).	Wang Z., et al. (2021); data collection date not reported
Cross-sectional	Women and men Chinese origin Mean age 59, $n = 96$	Stool samples; DXA	16s rRNA gene seq; potential pathway analysis; BMD	Differing bacteria negatively associated with OP depending on site; numerous KEGG pathways enhanced in OP.	Xu Z., et al. (2020); data collected 2019
Cross-sectional	Women Chinese origin Mean age 58, $n = 106$	Stool and blood samples; DXA	16s rRNA gene seq; BTMs; metabolomics; BMD	Several bacteria positively associated with OP, depending on site compared to healthy; adenosine and deoxyadenosine metabolites higher in OP than healthy.	He J., et al. (2020); data collected 2018–2019
Cross-sectional	Women Mexican origin Mean age 63, $n = 92$	Stool and blood samples; DXA; FFQ	16S rRNA gene seq; metabolomics; BMD	Higher abundance of *γ-Proteobacteria* in low-BMD group compared to normal BMD group; 4 amino acids lower in low-BMD group.	Palacios-Gonzalez B., et al. (2020); data collected 2018
Cross-sectional	Women and men Irish origin Mean age 64.5, $n = 181$	Stool and blood samples; DXA; FFQ	16s rRNA gene seq; BMD	Numerous bacteria positively and negatively associated with OP compared to healthy.	Das M. et al. (2019); data collection date not reported
Cross-sectional	Women and men Chinese origin Mean age 66, $n = 102$	Stool samples; DXA	16s rRNA gene seq; potential pathway analysis; BMD	Differing bacteria negatively associated with OP depending on site; 92 KEGG pathways differed in abundance between groups.	Li C., et al. (2019); data collected 2016–2017

Table 1 (continued)

Study design	Population, *n*	Exposure or intervention	Outcome measures	Results	References
Cross-sectional	Men US origin Mean age 84, *n* = 831	Stool samples; DXA, HR-pQCT; FFQ	16s rRNA gene seq; BMD; microarchitecture of radius and tibia	At FDR ≤ 0.1, higher abundance of *Anaerofilum* associated with low radial and tibial BMD and strength and with greater porosity, *Methanomassiliicoccus* associated with greater porosity, *Ruminiclostridium 9* associated with less porosity, and *Tyzzerella* associated with greater tibial BMD.	Orwoll E. S., et al. (2022); data collection started in 2015

RCT randomized controlled trial, *BMD* bone mineral density, *BM* bone mass, *BTM* bone turnover markers, *DXA* dual-energy X-ray absorptiometry, *LS* lumbar spine, *GM* gut microbiome, *OP* osteoporosis, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *FN* femoral neck, *FFQ* food frequency questionnaire, *HR-pQCT* high-resolution peripheral quantitative computed tomography

Gut Microbial Alpha and Beta Diversity

Alpha diversity is used to evaluate the richness and diversity of the gut microbiome within samples. Commonly reported indices include Chao1, Ace, Shannon, and Simpson. Beta diversity measures taxonomic dissimilarity between microbial samples with the most commonly reported indices including Bray-Curtis and weighted and/or unweighted UniFrac. The alpha and beta diversity findings from epidemiological studies included in this review are summarized in Table 2. The majority of studies reported no difference in alpha diversity between different states of bone health, one reported lower alpha diversity in those with poor bone outcomes, and three reported differing alpha diversity results depending upon the index used [15, 17–26]. Differences in beta diversity were more difficult to summarize due to varying author styles of reporting methods and data. Four studies reported no difference between different states of bone health while five reported a difference [15, 17–20, 22–25]. One study did not report beta diversity, one was not clear regarding the determination of this metric, and one additional study did not report either alpha or beta diversity results [16, 21, 26]. Standardization in reporting of gut microbial composition is needed for meaningful comparison of study findings. Inconsistencies in results across studies may be due to differences in the choice of index used to measure diversity or due to factors such as race and ethnicity, health status, age, sex, and medication use. The meta-analysis by Huang and colleagues reported no significant difference in Chao1 and Shannon indices between those with OP and healthy controls, both within and between races. Instead, they highlighted a variation in gut bacterial composition at the phyla (Bacteroidetes, Firmicutes) and genus levels (*Megamonas*, *Alistepes*, *Anaerostipes*, *Bacteroides*) associated with low BMD based on study geographical region [14••]. This study suggests that differences in geographical region may be responsible, in part, for the abundance of key gut microbiota observed in different states of bone health, and not race or ethnic group. Therefore, additional studies comprising diverse participants are necessary to compare differences in gut microbiome both by race/ethnic group and by geographic region.

Bone Turnover Markers

Two recent cross-sectional studies assessed the association between the gut microbiome and bone health via serum BTMs in adults [23, 25]. Both studies were conducted in older women of Chinese origin and reported different bacteria to be associated with measured BTMs. He and colleagues showed a statistically significant, positive correlation between the bone formation marker serum procollagen type 1 N-terminal propeptide (P1NP) and the bone

Table 2 Summary of alpha and beta diversity findings across epidemiological studies from the past 5 years

References	Method α diversity	No difference α diversity	$\downarrow \alpha$ diversity in OP	Differing results by method	Method β diversity	No difference β diversity	Difference in β diversity	Unclear or results not reported	Study results
Rettedal E. A., et al. (2021)	Shannon, Simpson	X			Bray-Curtis		X		Numerous bacteria positively and negatively associated with OP; several enhanced KEGG pathways in OP compared with healthy.
Wang Y. et al. (2022)	Shannon, Simpson, Chao1, Ace	X			Weighted and unweighted UniFrac	X			Higher relative abundance of *Firmicutes* and *Clostridiales* and BMD; positive correlation between GM gene numbers and BMD in women.
Wang Z.	Shannon, Simpson, Chao1, Ace			X	Bray-Curtis	X			Decreased proportion of *Prevotella* in OP (FN and LS) compared to osteopenia (~60%). Varying results with α diversity; no differences in β diversity.
Xu Z., et al. (2020)	Shannon, Simpson, Chao1, Ace			X	Weighted and unweighted UniFrac		X		Differing bacteria negatively associated with OP depending on site; numerous KEGG pathways enhanced in OP.
Palacios-Gonzalez B., et al. (2020)	Shannon, Chao1, observed species			X				X	Higher abundance of *γ-Proteobacteria* in low-BMD group compared to normal BMD group; 4 amino acids lower in low-BMD group.
He J., et al. (2020)	Shannon, observed species		X		OTU abundance		X		Several bacteria positively associated with OP, depending on site compared to healthy; adenosine and deoxyadenosine metabolites higher in OP than healthy.

Table 2 (continued)

References	Method α diversity	No difference α diversity	$\downarrow \alpha$ diversity in OP	Differing results by method	Method β diversity	No difference β diversity	Difference in β diversity	Unclear or results not reported	Study results
Greenbaum J., et al. (2022)	Not reported				Bray-Curtis			X	*Bacteroidetes* and *Fusobacteria* negatively associated with BMD; *Firmicutes* positively associated; several metabolic pathways in GM associated with BMD.
Das M., et al. (2019)	Shannon, observed species	X			Bray-Curtis			X	Numerous bacteria positively and negatively associated with OP compared to healthy.
Ling C.W., et al. (2021)	Shannon, Chao1, observed species	X			Bray-Curtis		X		Differing bacteria positively or negatively associated with OP depending on site, possibly through peptidase and transcription pathways.
Wei M., et al. (2021)	Shannon, Simpson, Chao1	X			Weighted UniFrac		X		*Bacteroidetes*-dominated microbiome positively associated with OP (LS); 10 KEGG pathways enhanced in OP (LS).
Li C. et al. (2019)	Shannon, Simpson, Chao1, Ace	X			OTU abundance	X			Differing bacteria negatively associated with OP depending on site; 92 KEGG pathways differed in abundance between groups.
Chen L. et al. (2021)	Shannon, Simpson, Chao1, Pielou's evenness, observed species, Faith's PD, Good's coverage	X			Bray-Curtis	X			Taxon-specific bacterial differences associated with CTX; no differences in taxa associated with BMD in OP compared to healthy.

Table 2 (continued)

References	Method α diversity	No difference α diversity	$\downarrow \alpha$ diversity in OP	Differing results by method	Method β diversity	No difference β diversity	Difference in β diversity	Unclear or results not reported	Study results
Orwoll E. S. et al. (2022)	Shannon	X			Bray-Curtis, weighted and unweighted UniFrac	X			At FDR ≤0.1, higher abundance of *Anaerofilum* associated with low radial and tibial BMD and strength and with greater porosity, *Methanomassiliicoccus* associated with greater porosity, *Ruminiclostridium 9* associated with less porosity, and *Tyzzerella* associated with greater tibial BMD.

OP osteoporosis, *OTU* operational taxonomic unit, *BMD* bone mineral density, *BM* bone mass, *BTM* bone turnover markers, *DXA* dual-energy X-ray absorptiometry, *LS* lumbar spine, *GM* gut microbiome, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *FN* femoral neck, *FFQ* food frequency questionnaire, *HR-pQCT* high-resolution peripheral quantitative computed tomography

resorption marker carboxy-terminal crosslinked telopeptide of type I collagen (CTX) and *Allisonella*, *Klebsiella*, and *Megasphaera* in women with post-menopausal osteopenia [25]. Chen and colleagues showed a significant, negative correlation of *Clostridiaceae* with CTX, while *Clostridium Ruminococaceae* was significantly positively correlated [23]. Chen and colleagues also showed a negative correlation of *Bacteroides* and *Clostridium* with P1NP and a positive correlation of *Chryseobacterium* and *Dehalobacterium* with P1NP. The authors observed no difference in microbial diversity among women defined as low versus high bone turnover (based on serum BTM levels). To define clear associations with the GM and BTMs, or differing associations among subgroups, more research is needed that is generalizable to men and other race and/or ethnic groups, as well as longitudinal studies that can assess changes in GM with changes in BTMs over time.

Bone Mineral Density and Taxon Abundance

All featured epidemiological studies either measured BMD using dual-energy X-ray (DXA) or conducted gut microbial analysis in participants who had been previously imaged. Nearly all studies reported an association in at least one measure of the GM and BMD, although the reported site of BMD varied. The association between the relative abundance of gut bacterial genera and BMD was recently reviewed by Huang and colleagues (2022). Using linear discriminant analysis effect size (LEfSe), the authors assessed the number of studies reporting statistical differences in the primary microbial strains for which contradictory results were reported between women with OP and those with low or healthy BMD. Two strains differed by race, including the g-*Lachnospira* which was found to be significantly enriched in Mexican post-menopausal women with low BMD, versus two Chinese cohorts in which post-menopausal healthy women were enriched with this genus. The second strain was *f_Peptostreptococcaceae* which was significantly enriched in a Chinese cohort of women with post-menopausal osteoporosis versus a New Zealand cohort in whom this strain was enriched in the healthy post-menopausal women [14••]. These results demonstrate that the direction of association of specific taxa with bone differ by race, suggesting that interventions to improve bone health by GM modification may need to be tailored to different racial and/or ethnic groups not only due to cultural differences, but also due to differing biological responses.

Three additional studies not included in the meta-analysis by Huang and colleagues and conducted in Chinese cohorts and a fourth conducted in a US cohort reported on additional taxa associations with BMD [15, 16, 23, 27]. Three of the four studies were published after the meta-analysis and that is likely the case with the fourth given its publication

date of November 2021. However, the time frame of publications included in the meta-analysis was not provided by the authors, or was a list of publications excluded from the analysis. The first study reported 11 differentially abundant taxa associated with increased BMD and 13 associated with decreased BMD in post-menopausal women [23]. In a separate study, four species of *Bacteroides* and *Fusobacterium ulcerans* were shown to be negatively associated with BMD, while *Clostridium leptum* and *Ruminococcus lactaris* (both in *Firmicutes*) were positively associated with BMD [16•]. Wang and colleagues reported similar results as those found in the meta-analysis: differences in *f_Peptostreptococcaceae* in women with OP compared to healthy controls [15]. Lastly, in a study of older men residing throughout the USA, the abundance of two genera were associated with BMD: *Anaerofilum* (lower BMD) and *Tyzzerella* (greater BMD) [27•].

Bone Mineral Density and Enrichment of Functional Pathways

Six studies published in the last 5 years have evaluated potential functional pathways as defined by a particular set of genes. The researchers utilized the Kyoto Encyclopedia of Genes and Genomes (KEGG) with one conducted in a Western population, and the others conducted in Chinese populations [15–20]. Carbohydrate or protein metabolism, environmental information processing (membrane transport, signal transduction, and signaling molecules and interaction), and genetic information processing (translation, transcription, folding, sorting, and degradation) are commonly enriched functional pathways in individuals with poor BMD [15, 17–20]. Numerous other KEGG pathways were correlated with bone outcomes, but were unique to their respective study, or shown to lose significance after correction for multiple testing [16•].

In conclusion, while differences in alpha and beta diversity were not consistently observed in the studies included in the present review, given the variety of diversity indexes utilized in the studies, these results are difficult to interpret confidently. Additional studies utilizing standardized methods and including diverse participants are necessary to make meaningful comparisons. In contrast, individual taxa may indicate presence or absence of OP in Asian adults [14••]. Additional research is needed to determine whether these taxa are mechanistically important in the development of OP and if so, whether they can be targeted for therapeutic treatment and whether they vary by sex and/or race and ethnicity. Due to the paucity of research examining enrichment in functional pathways and bone health outcomes, it is difficult to assess whether reported differences are due to race ethnicity, and/or other lifestyle and sociodemographic patterns.

Randomized Controlled Trials

Informed by findings from pre-clinical and epidemiological studies, four intervention studies have been conducted within the past 5 years (Table 1); each has examined the impact of administered probiotics on bone outcomes in women. In one study, the probiotic was additionally enriched with several bioactive nutrients [28•].

Bone Turnover Markers

A trial in post-menopausal Japanese women measured mean relative change from baseline of select BTMs as secondary outcomes in response to 24 weeks of ingestion of *Bacillus subtilis* C-3102 supplement or placebo [29]. Changes in urinary levels of the bone resorption marker N-terminal telopeptide/creatinine between baseline and 12 weeks were significantly lower in the intervention group compared to the placebo group (placebo = 23.3 ± 5.9%, C-3102 = 1.6 ± 6.3%; $p = 0.015$), resulting in a significant group-by-time interaction effect ($p = 0.033$). Changes in serum levels of the bone resorption marker tartrate-resistant acid phosphatase isoform 5b (TRAP-5b) between baseline and 12 weeks trended toward a significant decrease in the intervention group compared to the placebo group (placebo = 4.5 ± 2.8%, C-3102 = −4.8 ± 3.8%; $p = 0.054$). No significant change was observed in either BTM after 24 weeks. Neither the bone formation marker bone alkaline phosphatase (BAP) nor intact parathyroid hormone differed significantly by group over the study period. The 16s rRNA gene sequencing revealed a significant decrease in Chao1 and Shannon indices from baseline to 24 weeks in the C-3102 group. Probiotic supplementation also resulted in changes in relative abundance of 11 genera over time; however, these changes did not correlate with bone outcomes.

Two 12-month studies in post-menopausal Swedish women investigated the impact of daily supplementation with different probiotics on BTMs [30, 31]. The ProBone study investigated the effects of *Lactobacillus paracasei* DSM 13434 and two strains of *Lactobacillus plantarum* (DSM 15312 and DSM 15313) compared to placebo on BTMs. Results revealed no significant difference between groups over time in serum levels of osteocalcin (OC), P1NP, CTX, or NTX [30]. The ELBOW study compared the effects of *Lactobacillus reuteri* 6475 compared to placebo on BTMs. The authors showed no significant differences between the groups in serum NTX or BAP [31]. These two studies suggest that 12-month supplementation of probiotics had no effect on BTMs in northern European women.

Finally, a 6-month study in menopausal women of Spanish origin investigated the effects of daily intake

of a dairy product enriched with calcium, zinc, magnesium, vitamins D, C, and K, L-leucine, and *Lactobacillus plantarum 3547* on BTMs [28•]. Serum levels of P1NP were significantly increased from baseline in the women consuming the enriched dairy product, compared to controls (13.19 ± 25.17 ng/mL vs. −4.21 ± 15.62 ng/mL; $p < 0.05$), while levels of CTX were significantly decreased (−0.05 ± 0.19 ng/mL vs. 0.04 ± 0.14 ng/mL; $p < 0.05$). Although the strains used in Spanish women and in Swedish women were not identical, they were all from the same *Lactobacillaceae* genus.

Bone Mineral Density

BMD was also assessed in the four studies presented in this review that measured BTMs. In Japanese women, a significant increase in mean relative change total hip BMD was observed in those who consumed the C-3102 supplement (C-3102 = 2.53 ± 0.52%) compared to placebo (0.83 ± 0.63%; $p = 0.043$) but did not show a significant change in LS BMD. In contrast, results from the ProBone study showed no loss of LS bone in Swedish women after supplementation versus a significant loss of LS bone in those who received placebo (−0.72%, 95% CI: −1.22 to −0.22) [30]. Results from the ELBOW study revealed no significant difference in microarchitectural indices but did show a significant difference in mean relative change in tibia total volumetric BMD (1.02%, 95% CI: 0.02–2.03) between treatment and placebo. There were no significant differences in areal BMD at the total hip, femoral neck, or lumbar spine (L1–L4). Spanish women consuming an enriched dairy product maintained their BMD over the course of the study, compared to a significant loss of BMD in the placebo group (0.00 ± 0.00 g/cm^2 vs. −10 ± 10 g/cm^2; $p < 0.05$). While 30% of the intervention group was classified as osteopenic at baseline, only 24% were classified as such by the end of the study, compared to an increase in osteopenia in the placebo group, from 28 to 31%.

Overall, probiotic interventions yielded contrasting bone health results when assessed via BTMs. In women from Japan, intervention decreased a marker of bone resorption. A different probiotic did not impact markers of bone resorption in women from Sweden, but this same probiotic did improve markers of bone health in women from Spain. It is difficult to assess whether these contrasting bone responses are due to differences in probiotic strain, race or ethnicity, regional location, age, or mode of probiotic delivery (through enriched milk intake in the Spanish study, and pill supplementation in the Swedish studies). In contrast, supplementation with probiotics either maintained or increased BMD in women.

These studies highlight the paucity of racial and ethnic diversity as well as representation of men in clinical trials examining the GM and OP. As with the majority of the observational studies, the clinical trials are also limited by a lack of metagenomic analysis of the GM. A limitation of all observational studies discussed is their cross-sectional nature, which lack causality and are, therefore, hypothesis driving. Additionally, small sample sizes and a focus on women minimize generalizability to men and other subgroups. Future observational studies should be well-powered and longitudinal. Both observational and clinical studies would benefit from representation across sex and race ethnicity groups within and between different geographic regions, as well as including metagenomic sequencing of the gut microbiome.

Pre-clinical Studies

Mechanisms Underlying How the Gut Microbiome May Influence Bone

Studies in *Drosophila melanogaster*, livestock, and other mammals have shown the gut microbiota to be responsible for the synthesis of insulin-like growth factor 1 (IGF-1) through the production of short-chain fatty acids (SCFAs) in the gut [32, 33]. It has been suggested that SCFAs and other metabolic byproducts of gut microbial fermentation are absorbed into circulation and act on the liver and adipose tissue to induce the production of IGF-1. It is well established that IGF-1 is fundamental in skeletal growth, development, and maintenance throughout life [34]. Recent research also suggests that SCFAs act independently on bone, by operating directly on bone cell types such as osteoblasts, osteoclasts, chondrocytes, and fibroblasts, and indirectly, by stimulating antiinflammatory and immune regulatory responses [35]. Specific SCFAs may also be responsible for anabolism of bone tissue. For example, microbiota depletion in antibiotic-treated and germ-free mice resulted in lower butyrate levels [36]. When butyrate levels were reestablished, depleted parathyroid hormone (PTH) levels were restored, as well as number of bone marrow regulatory T cells (Tregs). This study suggests that adequate butyrate production in the gut is required for the anabolic action of PTH on bone.

The anabolic action of PTH on bone is suggested to occur via an attenuation of the oxidative stress that occurs as levels of sex steroids deplete with age and reactive oxygen species (ROS) accumulate. Dietary intake of antioxidants mitigates the deleterious effects of oxidative stress associated with inflammation-based diseases, and recent work in mice examined the effect of polyphenol supplementation (10% lyophilized blueberry, cultivar Montgomery) on skeletal endogenous antioxidant response [37]. Ovariectomized mice fed the blueberry-enriched diet exhibited significantly higher gut microbial α and β diversity, as well as significantly lower

percent change in BMD of the femur compared to controls. Results suggest that the blueberries exerted their protective effects against estrogen-induced bone loss via healthy gut microbial composition (significantly higher mean relative abundance of *Ruminococcus 1*, *Provotellacaea UCG-001*, and *Coriobacteriales Incertae* compared to controls) with a subsequent increase in EAR gene expression (*Hmox1*, *Ftl1*, *Gstp1*) in lumbar vertebrae of treated mice. Of note, these results were not observed consistently in orchiectomized males. While another study in 4-month-old ovariectomized rats supplemented with a 5% blueberry-enriched diet reported a 25.6% increase in bone calcium retention compared to control, a more recent study conducted on 5-month-old ovariectomized rats did not find an improvement in BMD or bone mechanical strength with 90 days of blueberry supplementation [38, 39]. The disparate outcomes reported in these studies may be explained by the different rodent species, the age of the animals studied, the length of the intervention, and/or the cultivar of blueberry.

The brain-gut-bone axis is of mechanistic interest due to the association of chronic stress and depression with OP [40]. Previous research has shown beneficial synergistic effects on this axis by supplementation with probiotics and the n-3 polyphenols eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) in a rat model with ligature-induced periodontitis [41]. Recent work investigated the effects of a diet comprising 0.6% EPA and 0.4% DHA with *Bifidobacterium longum*, *Lactobacillus helveticus*, and *Lactobacillus plantarum* (one group consumed live bacteria, the other dead bacteria) on stress-induced bone loss in 4-week-old male rats [42•]. Following 5 weeks of chronic mild stress, rats consuming the experimental diet exhibited significantly lower serum NTX, stress hormones (ACTH and cortisol), gut serotonin, and significantly higher brain serotonin, gut SCFAs (acetate, propionate, and butyrate), and BMD of the femur and tibia compared to rats consuming a non-supplemented diet in the face of stress. The 16s rRNA gene analysis of the GM revealed a significant increase in fecal abundance of *Lactobacillus* (~24%, $p = 0.026$) and *Blautia* (~5%, $p = 0.037$) between experimental and control animals, respectively. Consumption of live versus dead bacteria did not alter the additive effect of the combined EPA, DHA, and probiotic supplementation on outcomes.

In addition, recent evidence in mice suggests that the production of vitamin K by gut bacteria may be essential for the protection of bone tissue strength. Antibiotic-treated mice from 4 to 16 weeks of age showed significantly lower total cecal vitamin K compared to untreated mice and significant disruption of the gut microbiome was associated with decreased crystallinity (average difference 1.1%) [43]. In a separate study assessing which microbial contents influence bone tissue-level strength in mice, seven groups were treated with various antibiotic cocktails to selectively modify constituents of the gut microbiota [44•]. After 12 weeks of treatment, analysis of the fecal microbiota detected seven lower ranked taxa differentially abundant in animals with cortical bone tissue-level strength impairment, and 14 differentially abundant taxa associated with increased tissue-level strength. In addition, the only group to show impaired bone tissue strength also showed large reductions in many of the genes required to synthesize vitamin K, which is consistent with previous work in this area [45, 46]. Vitamin K is required for the synthesis of OC, and in the absence of this protein, bone tissue strength is altered [47, 48].

These studies provide potential mechanisms that can be tested in future human trials to elucidate differences in GM-OP outcomes across racial and ethnic groups. Skeletal differences such as hip axis length, size, bone thickness, and volumetric density are known to differ by sex and between racial and ethnic groups [49]. These differences are thought to contribute to differing fracture rates and could be the result of structural and/or functional differences in the GM. It is plausible that biological, environmental, and cultural differences by race ethnicity groups could cause one microbe to be beneficial in one group, yet pathogenic in another, resulting in differences in gut SCFAs, serotonin, and vitamin K levels, thereby influencing bone health [50]. For example, racial and ethnic differences in nutrient status, including vitamin D, have been reported to account for some of the racial and ethnic variation in diabetes and cardiovascular disease, findings that could extend to OP [51]. Shea and colleagues (2012) added to this body of literature by reporting racial and ethnic differences in vitamin K status which remained after adjustment for dietary, lifestyle, and sociodemographic covariates, suggesting that biological differences might explain the observed racial and ethnic differences in health outcomes such as bone strength that are related to vitamin K.

Conclusion

Examination of the recent literature investigating the pathway between the GM and bone health reveals a dominant focus on Asian adults, with modest consistency in the results. It is likely there is information to be gleaned from these studies that is applicable to other populations, but given the different outcomes reported in other populations and within the individual GM and OP fields, it is critical that the impact of race and ethnicity on this relation be examined more extensively. A recent meta-analysis of five continents showed Europe and Africa as having the highest prevalence of OP (18.6% and 39.5%, respectively), yet there are only a small number of recent studies examining GM and OP in cohorts from the former countries and none from the latter [1]. It is imperative that observational

studies and randomized controlled trials characterize the gut microbiome-bone health relation in men and women of diverse races and ethnic subgroups, paying particular attention to inclusion of underrepresented populations. In addition, comparison of race ethnic differences versus regional differences must be disentangled to better identify meaningful, culturally tailored interventions. Until these studies are conducted, any modification of the GM as a means of preventing or mitigating the effects of OP will represent a generalization of studies performed on homogeneous participants.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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