

Causal Relationship Between Gut Microbiota, Metabolites, and Sarcopenia: A Mendelian Randomization Study

Xiangyu Zhang, BM,^{1, } Guang Yang, MD,^{1,2} Shide Jiang, MD,³ Bingzhou Ji, MD,^{1,2}
Wenqing Xie, MD,^{1,2, } Hengzhen Li, MD,^{1,2, } Jianfeng Sun, MD,^{1,2} and Yusheng Li, PhD^{1,2,*}

¹Department of Orthopedics, Xiangya Hospital, Central South University, Changsha, China.

²National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, China.

³Department of Orthopedics, The Central Hospital of Yongzhou, Yongzhou, China.

*Address correspondence to: Yusheng Li, PhD. E-mail: liyusheng@csu.edu.cn

X.Z. and G.Y. contributed equally to this work.

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Abstract

Background: Gut microbiota imbalance and sarcopenia are frequently observed in older adults. Gut microbiota and their metabolites are considered risk factors contributing to the heightened risk of sarcopenia, but whether these associations are causal remains unclear.

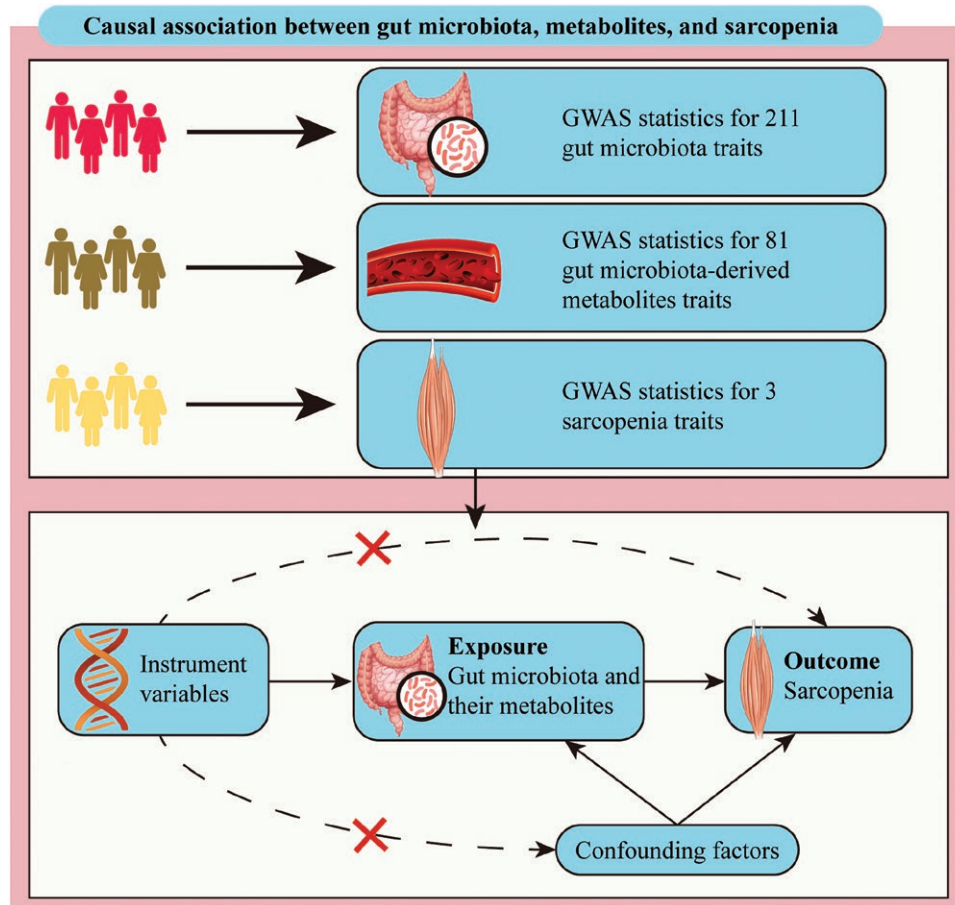
Methods: We conducted linkage disequilibrium score regression and 2-sample Mendelian randomization (MR) methods with single-nucleotide polymorphisms sourced from large-scale genome-wide association studies as instrumental variables to examine the causal associations linking gut microbiota with their metabolites to the sarcopenia. Following the MR analysis, subsequent sensitivity analyses were conducted to reinforce the robustness and credibility of the obtained results.

Results: MR analysis yielded compelling evidence demonstrating the correlation between genetically predicted gut microbiota and metabolites and the risk of sarcopenia. The abundance of *Porphyromonadaceae*, *Rikenellaceae*, *Terrisporobacter*, and *Victivallis* was found to be associated with walking pace. Our study also found suggestive associations of 12 intestinal bacteria with appendicular lean mass, and of *Streptococcaceae*, *Intestinibacter*, *Paraprevotella*, *Ruminococcaceae* UCG009, and *Sutterella* with grip strength. Specifically, we identified 21 gut microbiota-derived metabolites that may be associated with the risk of sarcopenia.

Conclusions: Utilizing a 2-sample MR approach, our study elucidates the causal interplay among gut microbiota, gut microbiota-derived metabolites, and the occurrence of sarcopenia. These findings suggest that gut microbiota and metabolites may represent a potential underlying risk factor for sarcopenia, and offer the promise of novel therapeutic focal points.

Keywords: Gastrointestinal microbiome, Mendelian randomization, Sarcopenia

Graphical Abstract



Sarcopenia is a degenerative and systemic condition that results in the loss of skeletal muscle mass and function, especially in the elderly population (1). Based on a comprehensive review and estimation of multiple research studies, it is evident that sarcopenia affects approximately 10%–16% of the global elderly population (2). Sarcopenia is intricately linked to an increased susceptibility to adverse events, including impaired functional capacity, falls, fractures, osteoporosis, metabolic syndrome, and diabetes (1,3,4). With the rapid progression of population aging, it is imperative to remain cautious of the escalating burden that sarcopenia imposes on healthcare and public health systems. About the cause of sarcopenia, there is a paucity of research elucidating its risk factors, underscoring the imperative for further exploration and uncovering (5). Evidence suggests that the composition and diversity of the gut microbiota may be determinants of skeletal muscle mass and function (6). Extensive research has already discovered the dysbiosis of the gut microbiota in populations with sarcopenia. And supplemental probiotics and fecal microbial transplantation have been shown to have therapeutic potential for sarcopenia in animal models (7,8). The gut–muscle axis suggests a complex interaction between gut microbiota and sarcopenia. However, there is still ample room for further exploration of its mechanisms and applications.

Compared to healthy individuals, patients with sarcopenia exhibit significant alterations in the composition and functionality of their gut microbiota (9). Gut dysbiosis primarily

disrupts metabolic pathways, including resistance to protein synthesis and downregulation of mitochondrial energy metabolism genes. Simultaneously, gut microbiota imbalance compromises the intestinal barrier, leading to the production of byproducts such as lipopolysaccharides and pro-inflammatory cytokines, resulting in chronic inflammation and immune dysfunction (10). Furthermore, the metabolites resulting from the interaction between gut microbiota and the host affect muscle synthesis, with the absence of beneficial metabolites and an increase in harmful byproducts observed following dysbiosis (11). These mechanisms may underlie the connection between the gut–muscle axis. However, the evidence establishing a causal relationship between gut microbiota and sarcopenia is limited. And the definition of sarcopenia has undergone evolution, resulting in varying cutoff criteria across different regions over the past decade (1,12). Results from observational studies and meta-analyses should be interpreted with caution due to the presence of residual confounding and measurement errors, which may introduce bias and contribute to the uncertainty surrounding causal relationships.

Mendelian randomization (MR) is an advanced epidemiological tool that utilizes genome-wide sequencing data to investigate causal relationships. By using genetic variants as instrumental variables, MR replaces traditional exposures and evaluates their causal associations with outcomes (13). The MR design offers the advantage of providing scientific evidence comparable to that of randomized controlled trials.

Significantly, the implementation of MR design circumvents the issue of reverse causation bias, as sarcopenia lacks the capability to exert an influence on an individual's genotype. Utilizing a 2-sample MR methodology, our research capitalizes on the largest genome-wide association study (GWAS) summary statistics to date. This robust approach facilitates the exploration of causal linkages among gut microbiota, metabolites, and phenotypes associated with sarcopenia.

Method

Data Sources and Study Participants

We selected the most comprehensive genetic data on gut microbiota available to date, which were obtained from the MiBioGen consortium. The data set of gut microbiota was obtained from a total of 18 340 participants, encompassing individuals of European, Hispanic, and Asian descent, among others. The study further categorized bacteria at the genus level and estimated the influence of human genetics on the presence and abundance of individual microbial taxa (14). Considering that gut microbiota-derived metabolites may play a more direct role in the interaction between bacteria and the host, we have incorporated gut microbiota-derived metabolites into our research. We acquired genetic data for 81 gut microbiota-dependent metabolite features from the Human Metabolome Database (HMDB) by utilizing a comprehensive GWAS summary data set that included data from TwinsUK and KORA, with a total of 7 824 participants (15,16).

Due to the lack of unified diagnostic criteria for sarcopenia, we have selected prevalent characteristics that can serve as representative indicators, encompassing walking pace (WP), appendicular lean mass (ALM), and low grip strength (GS). The GWAS on WP was derived from data on self-reported WP from 450 967 European ancestry participants in the UK Biobank (17). The UK Biobank database has amassed GWAS data from 450 243 individuals pertaining to ALM (18). The GWAS summary data for GS were derived from a meta-analysis of 256 523 individuals aged 60 years or older across 22 cohorts comprising individuals of European descent (19). Leveraging these population-based data sets, we conducted a genetic association study on variables associated with sarcopenia within the European population.

With the provided data, we utilized linkage disequilibrium score regression (LDSC) for quality control of GWAS summary data to detect the inflation of statistical quantities in GWAS data and ensure the reliability of our results (20) (Supplementary Tables 6 and 7). We conducted a 2-sample MR study to examine the causal connections between gut microbiota, metabolites, and 3 sarcopenia characteristics. The genetic variations under analysis must satisfy 3 crucial assumptions: (i) they exhibit an association with the exposure; (ii) they remain independent of confounding factors, unaffected by them; (iii) they exclusively relate to the outcome through the exposure and do not involve any other causal pathways (21). The flowchart of the study plan is shown in Figure 1.

Genetic Instrument Selection

According to the 3 requirements of MR analysis, independent single-nucleotide polymorphisms (SNPs) associated with the exposure at a genome-wide significance level ($p < 5 \times 10^{-8}$) were chosen as instrumental SNPs (in LD $r^2 = 0.001$ and kb = 10 000) (13). The corresponding association values for these

SNPs in the outcome data were obtained from their respective GWAS summary statistics. However, we observed that under this restriction, the majority of exposures had only 1 SNP available for study analysis, which could introduce errors. Therefore, we relaxed the criteria for SNP selection to 5×10^{-6} and conducted the analysis again (Supplementary Tables 1–3). The results obtained under these 2 different criteria were subsequently harmonized to generate the final results. Subsequently, we computed the F -statistic to assess the strength of the selected SNPs. A genetic SNP with an F -statistic >10 indicates good instrument strength, which can mitigate potential bias in MR analysis. For the second assumption of MR, we searched the PhenoScanner database (<http://www.phenoscanter.medschl.cam.ac.uk/>) to identify whether there were SNPs associated with confounding factors (body mass index, diabetes).

MR Analyses

The inverse variance weighted (IVW) method was employed as the primary statistical approach to assess the causal relationship between gut microbiota and sarcopenia. It is generally considered the most accurate method for estimating causality if there is no clear evidence of directional pleiotropy (p for MR-Egger intercept $> .05$). In cases where there was not enough evidence of heterogeneity among the selected instruments (p for MR heterogeneity $> .05$), a random-effects model was applied; otherwise, a fixed-effects model was assumed. Additionally, the weighted median method was performed, which can generate valid causal estimates when at least 50% of the selected instruments are valid SNPs. The MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test assessed pleiotropy and detected outliers. Subsequently, the IVW method was repeated.

Sensitivity Analyses

To validate our results, we performed a set of robustness checks. Cochran's Q statistic was utilized to assess heterogeneity among the instrumental variables. Furthermore, to detect horizontal pleiotropy, we also employed 4 MR methods (MR-Egger, weighted median, simple mode, weighted mode) and MR-PRESSO to examine the relationship between gut microbiota, metabolites, and the risk of WP, ALM, and GS. By combining these methods, we aimed to ensure the validity and reliability of our research findings to determine the potential causal relationship between gut microbiota, metabolites, and sarcopenia. All of the above analysis was done using the TwoSampleMR package (version 0.5.6) in R (version 4.3.0).

Ethical Approval and Consent for Participation

This study relies on publicly accessible data sets from GWAS and does not involve the use of individual-level data. Each study included in every GWAS was approved by the relevant Institutional Review Board, and prior informed consent was obtained from participants or their designated representatives (such as caregivers, legal guardians, or appointed proxies).

Results

Causal Estimates of Genetically Predicted Gut Microbiota on Sarcopenia

Following MR analysis, we found that family *Porphyromonadaceae* (odds ratio [OR] = 1.047, 95% confidence interval [CI] = 1.005–1.090, $p = 2.70\text{E-}02$, IVW), family *Rikenellaceae*

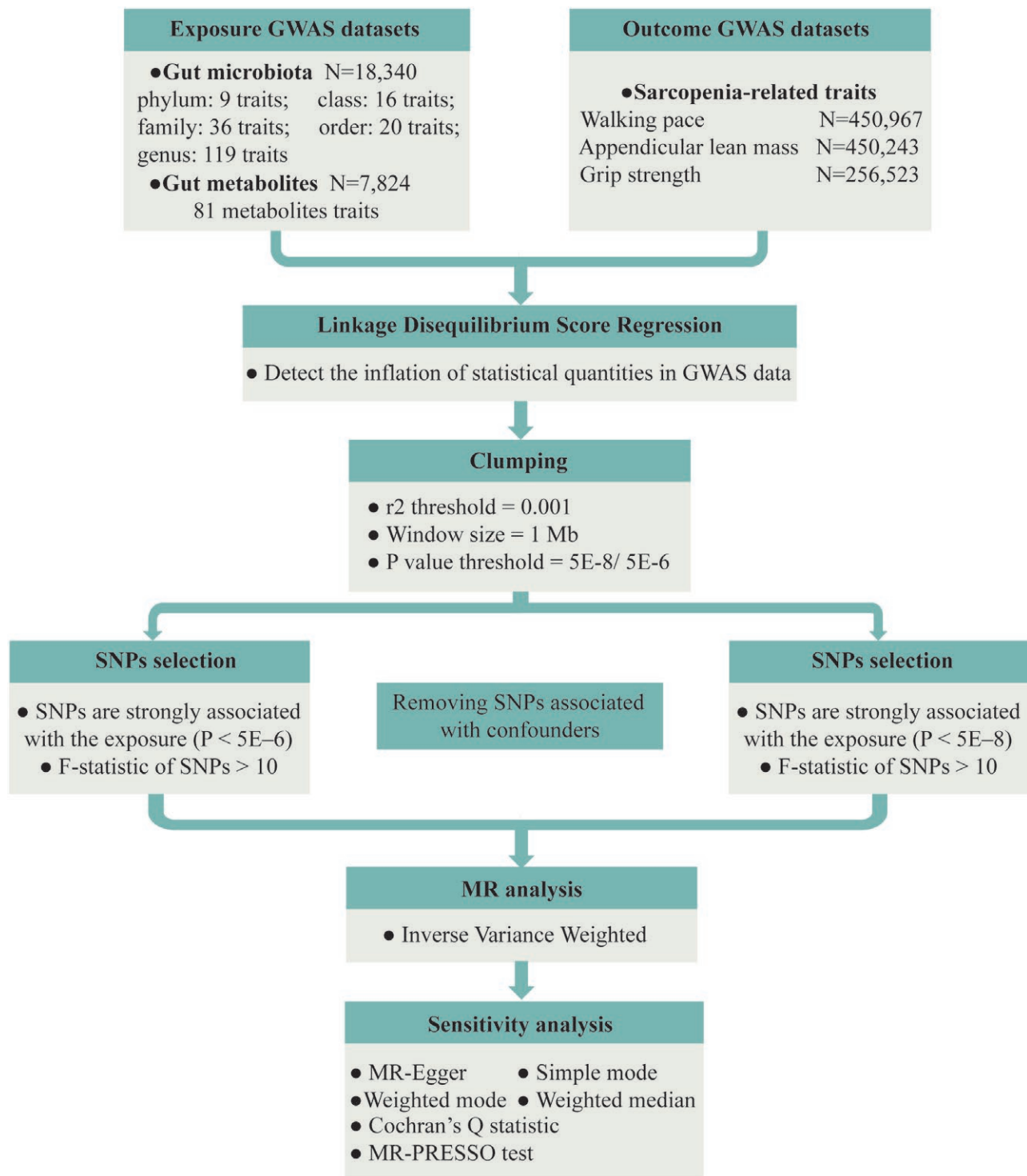


Figure 1. Flowchart for the study. GWAS = genome-wide association study; MR = Mendelian randomization; SNP = single-nucleotide polymorphism.

(OR = 0.984, 95% CI = 0.970–0.998, $p = 3.01E-02$, IVW), genus *Terrisporobacter* (OR = 1.019, 95% CI = 1.001–1.038, $p = 4.35E-02$, IVW), and genus *Victivallis* (OR = 1.011, 95% CI = 1.000–1.022, $p = 4.48E-02$, IVW) were causally associated with WP. And for ALM, we found class *Bacteroidia* (OR = 1.042, 95% CI = 1.005–1.079, $p = 2.37E-02$, IVW), family *FamilyXIII* (OR = 1.058, 95% CI = 1.023–1.095, $p = 1.16E-03$, IVW), genus *Erysipelotrichaceae* UCG003 (OR = 0.914, 95% CI = 0.878–0.952, $p = 1.71E-05$, IVW), genus

Eubacterium coprostanoligenes group (OR = 1.032, 95% CI = 1.003–1.061, $p = 2.99E-02$, IVW), genus *Gordonibacter* (OR = 1.020, 95% CI = 1.001–1.040, $p = 3.67E-02$, IVW), genus *Lachnospiraceae* NC2004 group (OR = 1.042, 95% CI = 1.015–1.069, $p = 2.22E-03$, IVW), genus *Oscillospira* (OR = 0.960, 95% CI = 0.929–0.991, $p = 1.29E-02$, IVW), genus *Phascolarctobacterium* (OR = 0.960, 95% CI = 0.931–0.989, $p = 7.59E-03$, IVW), genus *Ruminococcus2* (OR = 0.975, 95% CI = 0.953–0.997, $p = 2.86E-02$, IVW),

order *Bacteroidales* (OR = 1.042, 95% CI = 1.005–1.079, $p = 2.37\text{E-}02$, IVW), phylum *Firmicutes* (OR = 1.035, 95% CI = 1.001–1.070, $p = 4.37\text{E-}02$, IVW), and phylum *Lentisphaerae* (OR = 0.983, 95% CI = 0.967–0.999, $p = 3.74\text{E-}02$, IVW) were causally associated with it. Furthermore, we found that family *Streptococcaceae* (OR = 1.104, 95% CI = 1.006–1.211, $p = 3.72\text{E-}02$, IVW), genus *Intestinibacter* (OR = 1.136, 95% CI = 1.042–1.239, $p = 3.67\text{E-}03$, IVW), genus *Paraprevotella* (OR = 0.932, 95% CI = 0.881–0.986, $p = 1.50\text{E-}02$, IVW), genus *Ruminococcaceae* UCG009 (OR = 1.085, 95% CI = 1.004–1.172, $p = 3.95\text{E-}02$, IVW), genus *Sutterella* (OR = 0.850, 95% CI = 0.733–0.986, $p = 3.24\text{E-}02$, IVW) were causally associated with GS. In the sensitivity analysis, at least 1 method showed that family *FamilyXIII*, genus *Erysipelotrichaceae* UCG003, genus *Lachnospiraceae* NC2004 group, genus *Oscillospira*, genus *Ruminococcus2*, phylum *Lentisphaerae*, genus *Intestinibacter*, and genus *Sutterella* had the same direction as IVW, and the p value was statistically significant ($p < .05$; Figure 2; Supplementary Table 4).

Causal Estimates of Genetically Predicted Gut Microbiota-Derived Metabolites on Sarcopenia

Following MR analysis, we found that creatine (OR = 0.952, 95% CI = 0.911–0.995, $p = 2.76\text{E-}02$, IVW), benzoate (OR = 1.098, 95% CI = 1.004–1.201, $p = 4.00\text{E-}02$, IVW), carnitine (OR = 0.924, 95% CI = 0.867–0.985, $p = 1.55\text{E-}02$, IVW), malate (OR = 0.935, 95% CI = 0.877–0.996, $p = 3.80\text{E-}02$, IVW), mannitol (OR = 0.982, 95% CI = 0.965–1.000, $p = 4.41\text{E-}02$, IVW), and trans-4-hydroxyproline (OR = 1.113, 95% CI = 1.039–1.192, $p = 2.39\text{E-}03$, IVW) were causally

associated with WP. And for ALM, we found that acetylcarnitine (OR = 0.437, 95% CI = 0.229–0.834, $p = 1.20\text{E-}02$, IVW), creatine (OR = 1.369, 95% CI = 1.024–1.832, $p = 3.42\text{E-}02$, IVW), glycine (OR = 1.243, 95% CI = 1.057–1.460, $p = 8.35\text{E-}03$, IVW), ketoleucine (OR = 0.788, 95% CI = 0.640–0.970, $p = 2.49\text{E-}02$, IVW), lysine (OR = 0.677, 95% CI = 0.504–0.909, $p = 9.54\text{E-}03$, IVW), proline (OR = 1.112, 95% CI = 1.020–1.213, $p = 1.65\text{E-}02$, IVW), 4-hydroxyhippurate (OR = 1.070, 95% CI = 1.023–1.119, $p = 2.95\text{E-}03$, IVW), betaine (OR = 0.739, 95% CI = 0.568–0.960, $p = 2.37\text{E-}02$, IVW), deoxycholate (OR = 0.930, 95% CI = 0.885–0.977, $p = 3.75\text{E-}03$, IVW), glycodeoxycholate (OR = 0.974, 95% CI = 0.951–0.998, $p = 3.19\text{E-}02$, IVW), hippurate (OR = 0.925, 95% CI = 0.866–0.989, $p = 2.22\text{E-}02$, IVW), hyodeoxycholate (OR = 1.023, 95% CI = 1.000–1.046, $p = 4.55\text{E-}02$, IVW), phenylacetylglutamine (OR = 0.938, 95% CI = 0.887–0.992, $p = 2.63\text{E-}02$, IVW), and stachydrine (OR = 1.051, 95% CI = 1.008–1.097, $p = 2.04\text{E-}02$, IVW) were causally associated with it. Furthermore, we found that betaine (OR = 2.473, 95% CI = 1.648–3.711, $p = 1.23\text{E-}05$, IVW), glycine (OR = 0.752, 95% CI = 0.630–0.897, $p = 1.57\text{E-}03$, IVW), hippurate (OR = 1.271, 95% CI = 1.026–1.575, $p = 2.83\text{E-}02$, IVW), p-cresol sulfate (OR = 1.166, 95% CI = 1.003–1.356, $p = 4.62\text{E-}02$, IVW), and pyruvate (OR = 1.302, 95% CI = 1.031–1.645, $p = 2.67\text{E-}02$, IVW) were causally associated with GS. In the sensitivity analysis, at least 1 method showed that creatine and benzoate for WP, glycine, lysine, betaine, deoxycholate, and glycodeoxycholate for ALM, as well as betaine and glycine for GS, had the same direction as IVW, and the p value was statistically significant ($p < .05$; Figure 3; Supplementary Table 5).

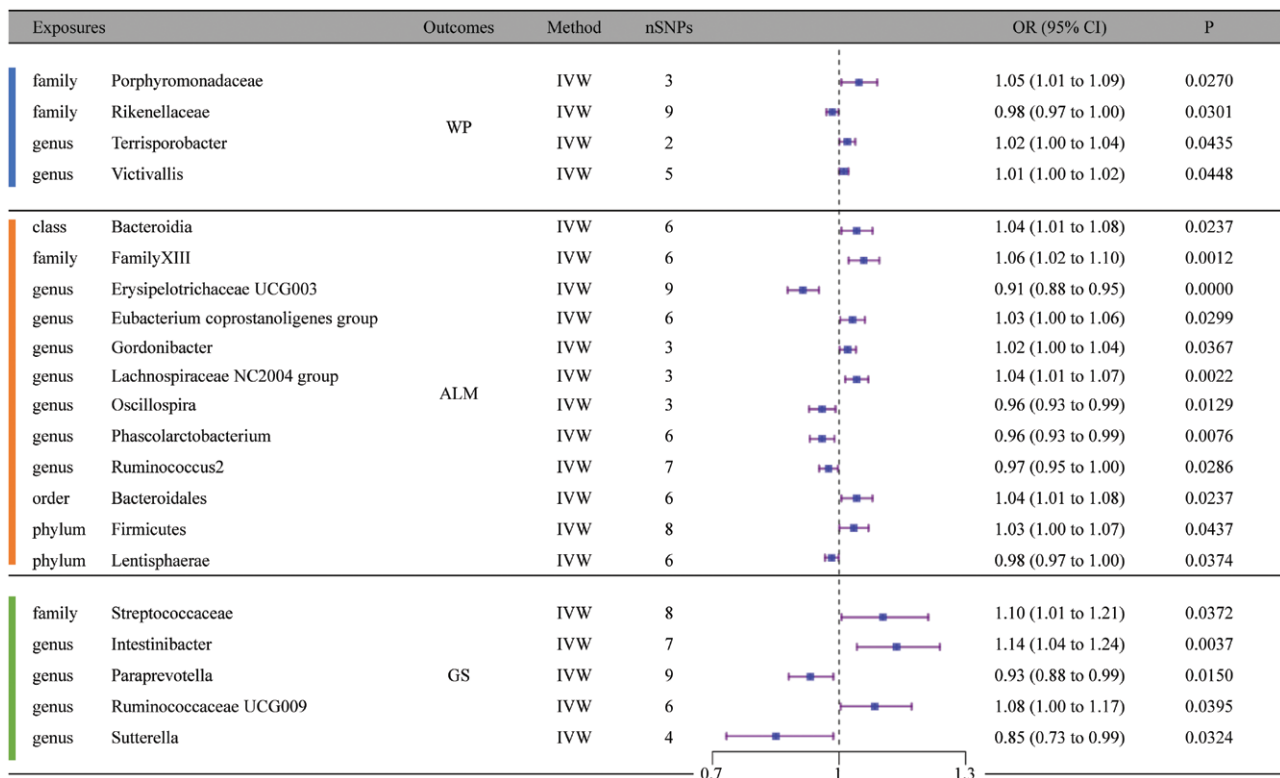


Figure 2. Mendelian randomization results of causal effects between gut microbiota and sarcopenia risk. ALM = appendicular lean mass; CI = confidence interval; GS = grip strength; OR = odds ratio; SNP = single-nucleotide polymorphism; WP = walking pace.

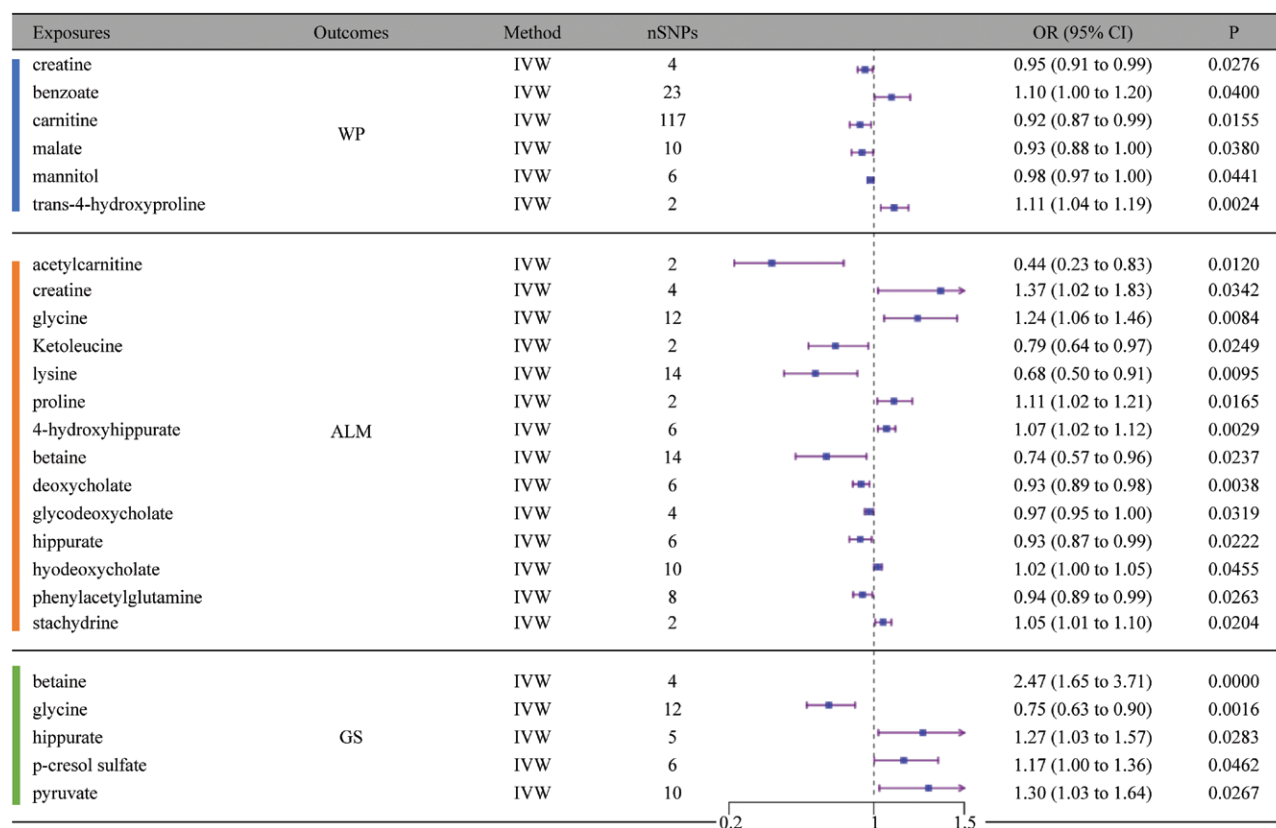


Figure 3. Mendelian randomization results of causal effects between gut microbiota-derived metabolites and sarcopenia risk. ALM = appendicular lean mass; CI = confidence interval; GS = grip strength; OR = odds ratio; SNP = single-nucleotide polymorphism; WP = walking pace.

Discussion

MR could be designed using existing large-scale GWAS data, overcoming the limitations of confounding factors that may bias traditional observational studies (22). Compared to case-control and cohort studies, MR provides a higher level of evidence, reflecting the lifelong impact of risk factors (23). The strengths of this study include utilizing the largest and most recent summary data from GWAS on gut microbiota and sarcopenia to investigate the unclear relationship of the gut-muscle axis. The HMDB, which is the largest and most comprehensive database of human metabolomics, was used to select metabolites derived from gut microbiota (15). The substantial sample size ensures the statistical power of the study results. Conducting high-quality MR analysis hinges on rigorous quality control of GWAS data. We employed LDSC as a method for data quality control, which helps distinguish inflation of statistical quantities in GWAS summary data (20).

Using MR analysis, we obtained a significant number of gene-level predicted associations between gut microbiota abundance and the phenotype of sarcopenia, further reinforcing our previous findings (24). GS and WP can serve as metrics for assessing muscle function. We have identified a positive causal relationship between *Porphyromonadaceae*, *Terrisporobacter*, *Victivallis*, *Streptococcaceae*, *Intestinibacter*, and *Ruminococcaceae* UCG009 and impaired muscle function. Meanwhile, *Rikenellaceae*, *Paraprevotella*, and *Sutterella* may act as protective factors for muscle function. Based on existing research, we have observed that while some of these microbes are not commonly mentioned in the context of sarcopenia, the majority of them are linked to fat. An observational

study conducted in an elderly population in Italy showed a significant association between a higher proportion of individuals with *Porphyromonadaceae* and *Rikenellaceae* and lower visceral fat (25). Similarly, an animal experiment indicated a negative correlation between *Victivallis* and obesity and hepatic steatosis (26). A meta-analysis reveals that in obese populations, the abundance of *Streptococcaceae*, *Paraprevotella*, *Sutterella* is higher compared to nonobese individuals. The increase in visceral fat and fat infiltration in skeletal muscles caused by obesity may be important pathogenic mechanisms for sarcopenia (27). ALM can serve as metrics for assessing muscle mass. We have identified that *Bacteroidia*, *FamilyXIII*, *Eubacterium coprostanoligenes* group, *Gordonibacter*, *Lachnospiraceae* NC2004 group, *Bacteroidales*, and *Firmicutes* are risk factors for muscle mass loss, while there is a positive causal relationship between *Erysipelotrichaceae* UCG003, *Oscillospira*, *Phascolarctobacterium*, *Ruminococcus2*, *Lentisphaerae*, and muscle mass. Metabolic dysfunction is considered the primary mechanism through which microbes are believed to be associated with muscle mass loss. Compared to other species, *Firmicutes* and *Bacteroidales* are closely associated with the production of short-chain fatty acids, and *Eubacterium coprostanoligenes* group is closely linked to cholesterol metabolism (28). These findings appear to further support previous research on the relationship between gut microbiota and sarcopenia. Individuals with frailty and cancer cachexia show a significant reduction in the relative abundance of *Paraprevotella* and increased *Intestinibacter* and *Ruminococcaceae* (24,29,30). An observational study found

a positive correlation between the relative abundance of *Bacteroides* and the severity of sarcopenia (31). Furthermore, we have observed the microbiota that do not align with the findings of other studies. A prospective cohort research has indicated a correlation between sarcopenia and an increase in the abundance of *Sutterella* (32). Animal experiments have also suggested a close association between *Sutterella* and the aging and inflammatory phenotypes in sarcopenia (33). The role of *Sutterella* in the occurrence and progression of sarcopenia, whether as a risk factor or a compensatory factor, still requires further investigation. And we observed that, although *Ruminococcus2* and *Ruminococcaceae* UCG009 both belong to the Ruminococcaceae family, they have opposite directions of risk for the occurrence of sarcopenia. This may suggest that more detailed species-level classification may be necessary for future research on microbial functions.

In fecal microbiota transplantation in clinical studies, a meta-analysis has summarized the therapeutic effects of 85 different diseases. Most of these studies have focused on gastrointestinal conditions, with limited research directly related to muscle health (34). The relationship between metabolites derived from gut microbiota and sarcopenia suggests that targeting these metabolites could be a more effective intervention strategy. We found a positive causal relationship between benzoate, trans-4-hydroxyproline, betaine, hippurate, p-cresol sulfate, and pyruvate and impaired muscle function while carnitine, creatine, glycine, malate, and mannitol are protective factors for healthy muscle function. Furthermore, we also found a positive causal relationship between 4-hydroxyhippurate, creatine, glycine, hydoxychole, proline, and stachydrine and muscle mass loss, while acetylcarnitine, betaine, deoxychole, glycodeoxychole, hippurate, ketoleucine, lysine, and phenylacetylglutamine had negative correlations. Yang et al. employed statistical methods to identify 45 significantly different metabolites in cancer cachexia patients (35). Among them, the metabolite that overlaps with our results are 4-hydroxyproline, carnitine, glycine, malate, creatine, glycochole, lysine, pyruvate, and phenylacetylglutamine. The recent focus of research lies in understanding how the gut microbiota regulates muscle physiology. Among these, short-chain fatty acids have been extensively studied, and other bacterial metabolites include bile acids, amino acid derivatives, and polyphenol-derived metabolites. Some studies have indicated that in cachexia patients, the reduced metabolism of most amino acids by the gut microbiota leads to amino acid loss, particularly in *Lachnospiraceae* and *Ruminococcaceae*. In turn, it has additional effects on the body, including the impact of aromatic amino acid derivatives like p-cresol and phenylacetate and branched-chain amino acids that maintain normal muscle metabolism (36,37). One of the functions of gut microbiota metabolism is to convert primary bile acids into secondary bile acids. Bile acid derivatives activate the bile acid receptor TGR5 to varying degrees, leading to skeletal muscle protein degradation, muscle mass loss, and mitochondrial dysfunction in skeletal muscles. It is glycodeoxychole that is strongly linked to the reduction in skeletal muscle volume in nonalcoholic fatty liver disease patients (32,38). It is interesting that a study has proposed the related metabolic indicators of benzoate and hippurate as biomarkers for frailty and elderly syndrome (39). While hippurate typically increases with age, it decreases in frail individuals. Whether it has a more specific connection with muscles is still a subject for further exploration. One point that must be emphasized

is that betaine, creatine, glycine, and hippurate seem to have opposing effects on muscle mass and function. This may be related to dose-response, and we need to consider pharmacology and toxicology. While they may promote an increase in muscle mass, it is essential to investigate whether they also have adverse effects on muscle function. This necessitates a careful exploration of dose parameters.

The relationship between the gut microbiome and its associated metabolome remains to be fully elucidated. Visconti et al. investigated the impact of gut microbiota on host systemic metabolism, revealing that 46% of circulating metabolites are associated with microbial taxa and their metabolic pathways. Many circulating metabolites exhibit significant heritability (40). Dodd et al. demonstrated that manipulating *C. sporogenes* at a genetic level significantly altered indolepropionic acid concentrations in mice by enhancing their ability to metabolize aromatic amino acids (41). This highlights the substantial potential of gut microbiota to modulate physiological states by regulating their derived metabolites. However, the network of relationships between gut microbiota and their associated metabolites is undoubtedly complex. The influence of gut microbiota on metabolites may extend beyond metabolic activities, affecting the transfer and absorption of metabolites by modulating intestinal barrier integrity rather than bioavailability. The high redundancy of metabolic pathways among different microbial species leads to an average sharing of 82% of microbial metabolic pathways between randomly paired unrelated individuals, with an average of 10 metabolic pathways associated with each circulating metabolite (40). The intricate cross-network relationships pose challenges in establishing causal relationships between specific microbial groups and particular metabolites. Liu et al. conducted an MR study on gut microbiota and their associated metabolites, identifying and replicating only 13 causal relationships between 12 microbes and 8 metabolic traits (42). This suggests that MR may not be the optimal method for screening causal relationships between gut microbiota and metabolites.

Alterations in the abundance of specific bacterial species in the gut of sarcopenia patients may influence clinical indicators associated with muscle mass and physical function through the combined effects of multiple metabolites, resulting in variations in various clinical outcomes (43). Existing research indicates that gut microbiota primarily regulates amino acids, short-chain fatty acids, bile acid derivatives, and anti-inflammatory metabolites, all of which affect muscle mass and function (32). Differential analysis among sarcopenia patients has revealed that key metabolic-related microbes including *Lachnospiraceae*, *Firmicutes*, *Subdoligranulum*, *Faecalibacter*, *Eubacteria coprostanoligenes* group, *Erysipelotrichaceae* UCG003, and *Ruminococcaceae* are associated with purine metabolism, arginine and proline metabolism, alanine and glutamate metabolism, butyrate metabolism, and histidine metabolism (44). Similarly, our findings identify metabolically active microbes such as *Erysipelotrichaceae* UCG003, *Eubacterium coprostanoligenes* group, *Firmicutes*, *Lachnospiraceae*, *Ruminococcaceae*, and *Ruminococcus*, which are linked to proline, glycine, glutamate, and alanine metabolism. The *Firmicutes* and *Bacteroidetes* are considered indicators of microbial dysbiosis in assessing sarcopenia and other diseases, as they regulate short-chain fatty acids, particularly butyrate, and glucose metabolism, while also exerting anti-inflammatory effects to

improve muscle mass and function (45,46). Furthermore, our results suggest a potential causal relationship between phenolic compounds and uremic toxins (4-hydroxyhippurate, acetylcarnitine, hippurate, p-cresol sulfate, and phenylacetylglutamine) with sarcopenia. This presents another promising area for investigation. According to existing research, certain microbes and metabolites identified in our study exhibit strong correlations. *Streptococcus* is a prominent producer of mannitol, while *Bacteroides* is a key bacteria involved in the production of p-cresol sulfate (47,48). And phenylacetylglutamine is predominantly derived from *Lachnospiraceae* and *Ruminococcaceae* (49). Moreover, *Sutterella* has been demonstrated to produce pro-inflammatory factors like lipopolysaccharides and play a role in age-related sarcopenia (32).

We observed that the causal association directions between certain exposure traits and different phenotypes of sarcopenia, such as betaine and glycine, may vary. Other research studies on sarcopenia have also demonstrated varied effects of exposures due to the diversity of outcome data (50). This discrepancy might be linked to the definition of sarcopenia, as we chose populations displaying anomalies in WP, ALM, and GS as representatives of the condition. Sarcopenia's definition has undergone multiple revisions in the past decade, and as of now, there exists no universally accepted diagnostic method for sarcopenia. The challenges in diagnosis present obstacles to the collection and statistical analysis of patient information (51). Individuals with diminished GS and reduced WP may not necessarily be afflicted with sarcopenia. Furthermore, those affected by the condition may manifest varying symptoms. Exploring whether there are significant differences in the composition of gut microbiota and their metabolites among populations with different symptoms is also a direction worth investigating. While patients may experience decreased muscle strength and mass, there could be notable differences in phenotypes such as GS and WP. Overall, there indeed exists a pronounced directional association between exposure and these distinct phenotypes. Further research using more advanced designs and methods to comprehensively explore the effects of these exposures is warranted.

Limitations

It is essential to acknowledge that this study comes with certain limitations. Firstly, the MiBioGen database includes populations from various genera, and the relationship between genes and the abundance of gut microbiota may have resulted in average outcomes by eliminating some population-specific effects. However, at least 72% of individuals in the gut microbiota GWAS cohorts are of European ancestry, and the statistical data for gut microbiota-derived metabolites and outcomes (WP, ALM, and GS) predominantly originate from individuals of European descent. Genetic variation is unevenly distributed across different ethnic or racial groups, which may restrict the generalizability of the study findings to specific ethnic populations (52). Therefore, further investigation is necessary to ascertain the applicability of our study results to specific ethnic populations. Additionally, the restricted number of SNPs employed in specific gut microbiota and metabolite MR analyses could lead to causal findings that lack robust support. It is essential to underscore that, akin to other MR studies, our research relies exclusively on genomic predictions to assess the linear causal impact of exposures on outcomes, reflecting the average effect of exposures throughout the lifespan. However, these associations

may be more intricate, displaying nonlinear relationships or susceptibility to environmental influences. Future directions could involve integrating genomic predictions with clinical studies or employing more sophisticated methodologies to investigate the specific roles of the microbiota and metabolites in the pathogenesis of sarcopenia. Furthermore, validating their potential as clinical biomarkers and therapeutic targets will require rigorous testing through randomized controlled trials.

Conclusion

The MR analysis provides compelling evidence that establishes a plausible causal nexus between gut microbiota, metabolites and susceptibility to sarcopenia. Strategically altering the abundance of gut microbiota and the concentrations of their correlated metabolites stands as a feasible approach in ameliorating the risk of sarcopenia. Overall, monitoring and modulating the gut microbiota is a preemptive strategy against the onset of sarcopenia, particularly in individuals characterized by perturbed gut microbiota and metabolite profiles.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None.

Data Availability

The data utilized in this study were sourced from publicly available summary-level data sets. Data on sarcopenia were obtained at GWAS Catalog (<https://www.ebi.ac.uk/gwas/>). The summary data derived from GWAS on gut microbiota (Kurilshikov et al.) can be accessed via <https://www.mibio-gen.org/>. The metabolomic GWAS summary statistics (Shin et al.) were downloaded from the metabolomics GWAS server (<https://metabolomips.org/gwas/index.php?task=download>).

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Author Contributions

X.Z. and G.Y. made equal contributions to data analysis, visualization, and manuscript writing. Y.L. conceptualized, methodologically guided, and supervised this work. S.J., B.J., and J.S. contributed to the statistical analysis. W.X. and H.L. were responsible for data validation. All authors contributed to data acquisition or interpretation, critically revised the manuscript for important intellectual content, and gave final approval for publishing the version.

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