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Prevotella copri alleviates sarcopenia via attenuating muscle mass loss and function decline

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Abstract

Background The gut microbiome and fecal metabolites have been found to influence sarcopenia, but whether there are potential bacteria that can alleviate sarcopenia has been under-investigated, and the molecular mechanism remains unclear.
Methods To investigate the relationships between the gut microbiome, fecal metabolites and sarcopenia, subjects were selected from observational multi-ethnic study conducted in Western China. Sarcopenia was diagnosed according to the criteria of the Asian Working Group for Sarcopenia 2014. The gut microbiome was profiled by shotgun metagenomic sequencing. Untargeted metabolomic analysis was performed to analyse the differences in fecal metabolites. We investigated bacterium with the greatest relative abundance difference between healthy individuals and sarcopenia patients, and the differences in metabolites associated with the bacteria, to verify its effects on muscle mass and function in a mouse model.
Results The study included 283 participants (68.90% females, mean age: 66.66 years old) with and without sarcopenia (141 and 142 participants, respectively) and from the Han (98 participants), Zang (88 participants) and Qiang (97 participants) ethnic groups. This showed an overall reduction (15.03% vs. 20.77%, $P = 0.01$) of *Prevotella copri* between the sarcopenia and non-sarcopenia subjects across the three ethnic groups. Functional characterization of the differential bacteria showed enrichment (odds ratio = 15.97, $P = 0.0068$) in branched chain amino acid (BCAA) metabolism in non-sarcopenia group. A total of 13 BCAA and their derivatives have relatively low levels in sarcopenia. In the in vivo experiment, we found that the blood BCAA level was higher in the mice gavaged with live *P. copri* (LPC) ($P < 0.001$). The LPC mice had significantly longer wire and grid hanging time ($P < 0.02$), longer time on rotor ($P = 0.0001$) and larger grip strength ($P < 0.0001$), indicating better muscle function. The weight of gastrocnemius mass and rectus femoris mass ($P < 0.05$) was higher in LPC mice. The micro-computed tomography showed a larger leg area ($P = 0.0031$), and a small animal analyser showed a higher lean mass ratio in LPC mice ($P = 0.0157$), indicating higher muscle mass.
Conclusions The results indicated that there were lower levels of both *P. copri* and BCAA in sarcopenia individuals. In vivo experiments, gavage with LPC could attenuate muscle mass and function decline, indicating alleviating sarcopenia. This suggested that *P. copri* may play a therapeutic potential role in the management of sarcopenia.

Keywords Brand chain amino acid; Gut microbiome; *Prevotella copri*; Sarcopenia; Serum metabolome

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Introduction

Sarcopenia is common in the elderly and is characterized by reductions in both the mass and function of skeletal muscles, leading to decreased physical fitness and quality of life, falls, disability, and possible death in geriatric patients.[1] The prevalence of sarcopenia ranges from 9.90% to 40.40% in elderly communities.[2] As the pathogenesis of sarcopenia is not fully understood, research is required to elucidate the underlying mechanisms and develop effective interventions for the disorder.

The roles played by the gut microbiota in human health have been increasingly recognized, including the "gut-muscle axis" between the gut microbiome and skeletal muscles.[3] It has been found that the gut microbiota and its metabolites impact the muscles in various ways by influencing nutrient absorption, immune inflammation, and energy metabolism. Metabolites of the gut microbiota, including folic acid, vitamin B2, vitamin B12, betaine, tryptophan, short-chain fatty acids, and urolithin have been found to positively influence skeletal muscle mass and function by different mechanisms.[4] In contrast, metabolites such as indoxyl sulfate and p-cresol sulfate produced by the gut microbiota are negatively correlated with skeletal muscle mass and function.[5,6] Treatment of sarcopenia with probiotics has further demonstrated the potentially protective role of the gut microbiota in sarcopenia. However, the specific relationships between the gut microbiota and sarcopenia are still unclear and further investigation into the complex molecular mechanisms of this axis is required.

An animal study observed that the transplantation of pathogen-free gut microbiota into germ-free mice increased oxidative metabolism in the muscles of the recipients, resulting in increased mass and reduced atrophy.[7] Another cohort study observed that increased synthesis of the short-chain fatty acid butyrate by gut microbes led to raised levels of butyrate in the serum and an improved skeletal muscle index.[8] [Correction added on 11 September 2023, after first online publication: In the preceding sentence, the term 'animal study' has been corrected to 'cohort study' in this current version.] Moreover, the administration of a prebiotic composed of inulin plus fructo-oligosaccharides was shown to significantly improve exhaustion and handgrip strength in elderly patients.[9] However, most current studies have been conducted on animals with little consistency in the type, dose, or duration of the probiotic treatment, making it difficult to integrate research findings.

A systematic multi-omics data integrated analysis is necessary to study the associations among the gut microbiome, fecal metabolites, and muscle function. In addition, we were also curious about which bacteria are responsible for attenuating sarcopenia and thus explored the underlying molecular mechanisms. Here, we used shotgun metagenomic sequencing, untargeted metabolomics, and an animal model to investigate the influence of the gut microbiome and its metabolites on sarcopenia.

Methods

The West China Health and Aging Trend cohort and sarcopenia screening

.09pt?>To investigate the relationships among the gut microbiome, fecal metabolites and sarcopenia, we collected stool samples from both sarcopenia and healthy individuals over 50 years old in the West China Health and Aging Trend (WCHAT) aging cohort.[10] Sarcopenia was defined according to the criteria of the Asian Working Group for Sarcopenia 2014. Muscle mass was measured by the bioimpedance analysis method using an INbody720 body composition instrument (cut-off values of the appendicular skeletal muscle mass index were 7.0 kg/m^2 for men and 5.7 kg/m^2 for women). Grip strength was measured using a dynamometer (EH101; Camry, Zhongshan, China) to test the muscle strength (cut-off value of 26 kg for men and 18 kg for women). Gait speed was measured by walking speed over four meters, with speeds below 0.8 m/s defined as associated with sarcopenia.

Study participants and fecal collection

This study used the baseline data of WCHAT study. In this study, the inclusion criteria comprised (i) aged 50 years or older, (ii) had lived in the present locality for at least 36 months, (iii) no communication difficulties and able to complete a 50-min interview. Briefly, the exclusion criteria comprised (i) antibiotic treatment during the previous 3 months, (ii) the presence of serious diseases, including heart, liver, and kidney disease, and respiratory failure, (iii) life expectancy $<$6 months. Detailed information on the study design has been described previously.[10] We used a special collection container provided by the Beijing Quantitative Health Co., Ltd. company for stool. This contained a solution for preventing the degradation of DNA. Among the DNA solution, 0.15–0.35 mol/L disodium ethylenediaminetetraacetic acid, sodium chloride and 12–28% dimethyl sulfoxide are dissolved, and the pH is adjusted to 6.0–9.0. Fecal samples were obtained from all recruited subjects for metagenomic sequencing and non-targeted metabolomic detection. The methods for DNA extraction and DNA library construction are provided in the Supplementary Methods. The methods for sample preparations of metabolomic detection and metabolite profiling are also provided in the supporting information.

Metagenomic data analysis

Taxonomic profiles were characterized by Humann3.[11] The α-diversity (Shannon index) and β-diversity (Bray–Curtis dis-

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tance) were calculated using the vegan 2.6-4 package in R. Principal coordinate analysis (PCoA) was performed on the sample pairwise Bray–Curtis dissimilarity measures derived from the relative abundance table of the taxa. The permutational multivariate analysis of variance test was performed in vegan with 100 permutations to assess the significant factor for explaining the variation in the gut microbial samples. Enrichment of the abundant taxa between groups was performed using the linear discriminant analysis effect size (LEfSe, Galaxy Version 1.0) with an LDA log score cut-off of 2.[12] To further confirm the difference assessed by LEfSe, we also performed two-tailed unpaired Mann–Whitney *U* tests. Considering the effects of potential confounders, such as age, gender and ethnicity, a multivariable association analysis was performed using the linear model in R. The plots were visualized using the ggplot2 package in R and modified from EasyAmplicon.[13]

To investigate the functional attributes of gut microbiome, valid reads were de novo assembled into contigs for each sample. All coding regions (CDS) of metagenomic contigs were predicted by MetaGeneMark v3.26.[14] CDS sequences of all samples were clustered by CD-HIT v4.6.1 to obtain unigenes. The lowest common ancestor taxonomy of unigenes was obtained by aligning them against the NCBI nr database by DIAMOND v 0.9.14.[15] Mann–Whitney *U* test was carried out in R to find the differential genes, and the *P*-value was adjusted by the Benjamini–Hochberg procedure (adjusted *P*-value < 0.05). After that, KEGG pathway enrichment analysis was performed using the clusterProfiler v4.0 package (adjusted *P*-value < 0.05)[16] in R.

Data analysis of metabolites profiling

The online Human Metabolome Database 4.0 was used to annotate the metabolites by matching the exact molecular mass data (m/z) of samples with those from database. Information on the procedures after quality control was provided in the supporting information.

Supervised partial least squares discriminant analysis (PLS-DA) was conducted through metaX[17] to discriminate the different variables between groups. The variable important for the projection value and coefficient variable were calculated. Two-tailed unpaired Mann–Whitney *U* tests were conducted to detect differences in metabolite concentrations between healthy individuals and sarcopenia patients. The Benjaminin–Hochberg procedure was used to adjust *P* values. To identify the differential metabolites between healthy individuals and sarcopenia patients, the criteria were (i) variable important for the projection > 1.0, (ii) coefficient variable < 0.3, (iii) ratio > 1.5 and (iv) adjusted *P*-value < 0.05.

Bacteria strains, culture and preparation of bacteria suspensions

We chose *P. copri* for the animal experiment as it was the most differential species in healthy individuals compared with sarcopenia patients. *P. copri* (DSM 18205) were purchased from Beijing Biobw Biological Technology Co. Ltd. (Beijing, China) and cultured in anaerobically sterilized PYG liquid medium containing and Vitamin K1 under strict anaerobic conditions overnight. The bacteria were cultured in PYG liquid medium (Qingdao hopebio Technology Co. Ltd., Qingdao, China) under anaerobic conditions. Bacterial suspensions were washed with sterile PBS, centrifuged and resuspended in PBS to an OD600 = 1, which corresponds approximately to 5×10^8 colony forming units (CFU)/100 μL.

Function validation of Prevotella copri in mice

Twenty-month-old female C57BL/6 mice (Beijing Hua Hukang company) were housed in groups of five mice per cage (filter-top cages), with free access to food and water under a strict 12-h/12-h light/dark cycle. Based on body weight, they were randomly divided into four groups, namely, groups gavaged with sterile PBS, branched chain amino acid (BCAA), live *P. copri* (LPC) and heat-killed *P. copri*, respectively, with *P. copri* doses of 5×10^8 CFUs/100 μL in sterile PBS, respectively, three times a week for 8 weeks.

All the animal behaviour tests were measured before gavage and at the end of the gavage experiment. The primers of RT-PCR could be seen in Table S3. The animal composition analysis and micro-computed tomography scan were tested after the gavage. The specific procedures of all the above tests were provided in the supporting information. The stool samples were collected per each cage before gavage and at the end of the gavage experiment and immediately stored at −80°C before further analysis. The specific procedure of tissue collection, blood BCAA measurement, gene expression analysis, quantification of the abundance of *P. copri* in feces and Sirius Red staining experiments could be seen in the supporting information.

All procedures were approved by the Animal Care Committee of West China Hospital, Sichuan University (No. 20220704003). All the animal experiments were done in the State Key Laboratory of Biotherapy, Cancer Center/Collaborative Innovation Center for Biotherapy and Animal Center, West China Hospital, Sichuan University.

Data analysis of mouse study

Mice study data were represented as means ± standard error of the mean. GraphPad Prism version 7.0 was used for image

presentation. All statistical analyses were conducted in R version v4.1.3. Comparisons among four groups were assessed using one-way ANOVA for normally distributed data and Wilcoxon rank sum test for non-normally distributed data. Significance was set $P < 0.05$. The criteria for outlier elimination were as follows: For normally distributed data, any values outside the range of the mean ± 2 standard deviations were removed. For non-normally distributed data, any values outside the range of the quarter digit − 2IQR and quarter digit + 2IQR were removed.

Results

Characteristics of participants

To study the relationships among the gut microbiome, fecal metabolites and sarcopenia, we randomly selected 283 participants, balanced between those with sarcopenia and healthy controls, from the WCHAT aging cohort. In terms of ethnicity, 98 of the participants were of Han ethnicity, 88 were Zang and 97 were Qiang. The WCHAT cohort included 7536 participants covering three ethnic groups (Han, Qiang and Zang) spread over Western China.[10] Sarcopenia was defined according to the criteria of the Asian Working Group for Sarcopenia 2014. The characteristics of the subjects are shown in Table 1.

The mean age was 66.66 years old, and 68.90% of the participants were female. Compared with subjects without sarcopenia, sarcopenia sufferers tended to be older (69.10 ± 8.00 vs. 64.10 ± 9.20 years old), with lower body mass index (22.50 ± 2.70 vs. 26.20 ± 3.40 kg/m^2), lower quality-of-life scores on the independent activity daily life scale (13.00 ± 1.90 vs. 13.60 ± 1.10 scores) and the social support rate scale (41.00 ± 6.70 vs. 42.80 ± 7.10 scores). There was also a significant reduction in the albumin level in the sarcopenia group (44.00 ± 2.90 vs. 44.80 ± 2.70 g/L). Lastly, consistent with the decreased muscle mass and function characteristic of sarcopenia, individuals in the sarcopenia group showed significant reductions in grip strength (17.50 ± 6.50 vs. 18.70 ± 5.10 kg) and in skeletal mass index (SMI) values (5.60 ± 0.70 vs. 6.0 ± 0.80 kg/m^2) (Figure 1A).

Gut microbiome composition in the Han, Qiang and Zang ethnic groups

In our permutational multivariate analysis of variance analysis, we found that the ethnic group had a significant effect (F = 3.88, P-value = 0.01) on gut microbiome. In WCHAT cohort, we found that sarcopenia was prevalent in three ethnic

Table 1 Comparisons of demographic characteristics, anthropometric measures, life-styles, chronic diseases of the study participants with and without sarcopenia

Characteristics	No sarcopenia (n = 142)	Sarcopenia (n = 141)	P-value
Age, year, mean (±SD)	64.1 (9.2)	69.1 (8.0)	<0.001
Gender, N (%)			
Male	37 (26.1)	51 (36.2)	0.073
Female	105 (73.9)	90 (63.8)	
Ethnics group			0.325
Han, N (%)	43 (30.3)	55 (39.0)	
Zang, N (%)	46 (32.4)	42 (29.8)	
Qiang, N (%)	53 (37.3)	44 (31.2)	
Anthropometric measures			
BMI (kg/m^2), mean (±SD)	26.2 (3.4)	22.5 (2.7)	<0.001
Grip strength (kg), mean (±SD)	18.7 (5.1)	17.5 (6.5)	0.077
Gait speed(m/s), mean (±SD)	0.8 (0.2)	0.7 (0.2)	0.139
SMI (kg/m^2), mean (±SD)	6.7 (0.8)	5.6 (0.7)	<0.001
The quality of life			
ADL score, mean (±SD)	99.1 (2.7)	99.0 (3.0)	0.839
IADL score, mean (±SD)	13.6 (1.1)	13.0 (1.9)	0.003
SSRS score, mean (±SD)	42.8 (7.1)	41.0 (6.7)	0.026
Blood tests			
Albumin (g/L), mean (±SD)	44.8 (2.7)	44.0 (2.9)	0.019
Vitamin D (ng/mL), mean (±SD)	18.0 (5.9)	17.1 (5.6)	0.173
Haemoglobin (g/L), mean (±SD)	146.4 (17.1)	146.3 (17.7)	0.960
Creatinine (μmol/L)	81.0 (20.1)	79.1 (14.8)	0.391
Blood uric acid (μmol/L)	328.4 (84.0)	316.0 (80.3)	0.217
Blood urea nitrogen (mmol/L)	5.7 (1.9)	5.4 (1.4)	0.184

Clinical and demographic parameters of enrolled subjects. Data are shown using % or mean (standard deviation). P values were calculated with χ^2 tests and Student's t tests for categorical and continuous variables, respectively.
ADL, Activity of Daily Living Scale; BMI, body mass index; IADL, independent activity daily life scale; SD, standard deviation; SSRS, social support rate scale.

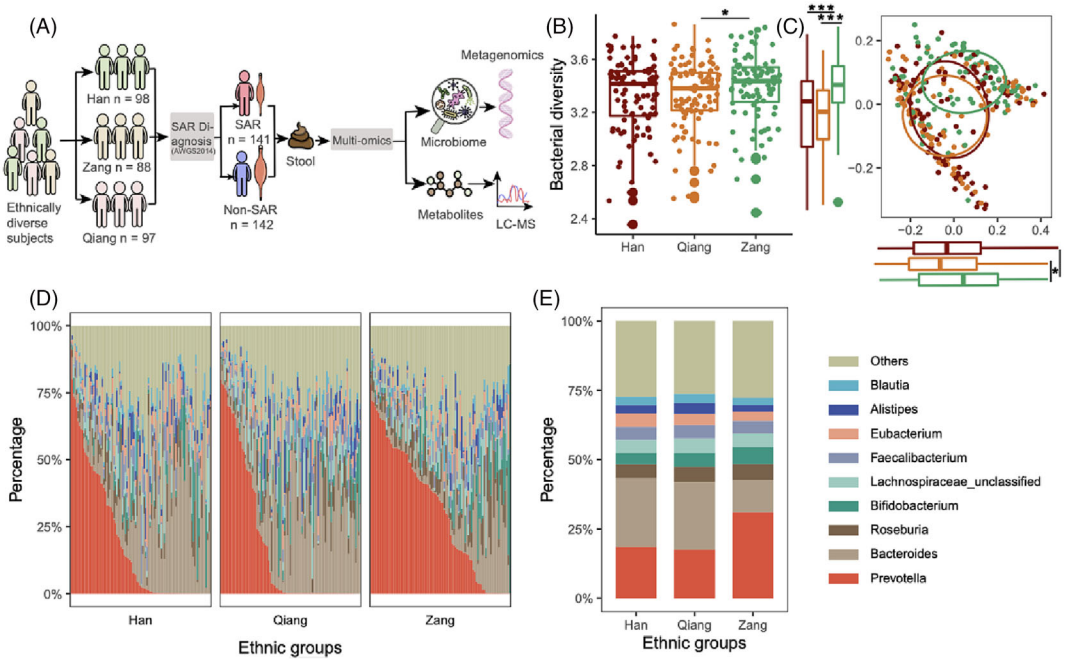

Figure 1 Ethnics and gut microbiome. (A) Study sampling and participant flow diagram. (B) Shannon index of the gut microbiome from Han, Qiang and Zang ethnic groups. The horizontal bars within boxes represent medians. The tops and bottoms of boxes represented the 75th and 25th percentiles, respectively. The upper and lower whiskers extended to data no more than 1.5 × the interquartile range from the upper edge and lower edge of the box, respectively. (C) Unconstrained PCoA (for principal coordinates PCo1 and PCo2) with Bray–Curtis distance showed that the gut microbiome of Zang separate from Han and Qiang in the first axis and the second axis. Ellipses covered 50% of the data for each ethnic groups. (D, E) Genus-level taxonomic distribution of the gut microbiome among ethnic groups at individual level (D) and on average (E). *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$; Wilcoxon rank-sum test.

groups.[18] Therefore, we first investigated the microbiome community structures in the three different ethnic groups. Our analysis showed that the Zang ethnic group had a distinct microbiome composition compared with individuals of Han and Qiang ethnicity. α-Diversity was higher in the Zang group (Figure 1B), and there was a clear separation between Zang individuals and the other two ethnic groups on the PCoA plots where the differences on the PCo1 and PCo2 axes were both significantly different (Figure 1C), indicating a more distinct community structure.

In terms of specific taxonomy, we observed some shared features. Specifically, *Prevotella*, *Bacteroides*, *Roseburia*, *Bifidobacterium*, *Lachnospiraceae*, *Faecalibacterium*, *Eubacterium*, *Alistipes* and *Blautia* were major bacterial genera in all the three ethnic groups. *Prevotella* and *Bacteroides* represented the two most abundant genera in microbiomes over all three ethnic groups (Figure 1D,E).

LEfSe also identified different gut bacteria in the three ethnic groups. Compared with Han and Qiang, the Zang group had a greater abundance of *Prevotella* sp. CAG:5226, *Firmicutes* bacterium CAG:170, *Roseburia* sp. CAG:309, *Lactococcus raffinolactis*, *Bacteroides* sp. CAG:530, *Ruminococcus* sp. CAG:563, *Firmicutes* bacterium CAG:238 and *Enterococcus durans*, while compared with the Han and Zang groups, the Qiang group showed greater numbers of *Alistipes finegold*, *Anaerostipes hadrus*, *Eggerthella lenta*, *Phascolarctobacterium faecium*, *Firmicutes* bacterium CAG:145 and *Anaerotruncus colihominis*. Furthermore, compared with both Qiang and Zang, Han individuals had higher levels of *Firmicutes* bacterium CAG:95 (Figure 2A,B). In summary, these findings demonstrated both shared and specific features of gut microbiome across the Han, Zang and Qiang groups.

Prevotella is less abundant in patients with sarcopenia across three ethnic groups

As the gut microbiome can vary from one ethnic group to another, we wanted to identify a common bacterium that can affect sarcopenia across all the three ethnic groups. To characterize the differences of the gut microbiome of sarcopenia patients and healthy individuals, we compared their ecological parameters and identified different taxa. At the ecological level of the whole gut microbiome community, there was no difference between sarcopenia

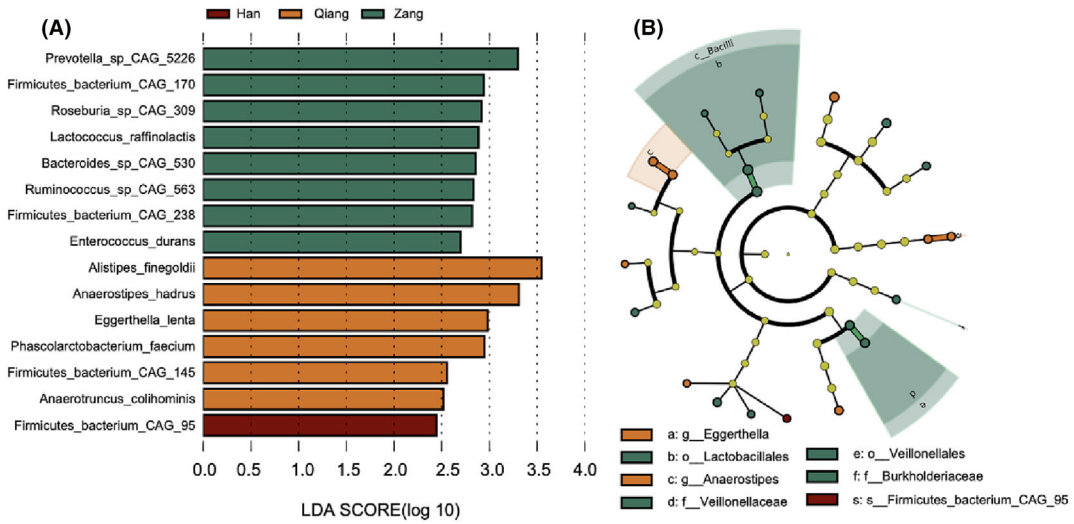

Figure 2 Gut microbiome differences of three ethnic groups. (A) LDA scores of the differentially abundant taxa in gut microbiome from three ethnic groups (taxa with LDA score > 2 and a significance of a < 0.05 are shown). (B) Cladograms, generated from LEfSe analysis, represented taxa enriched in gut microbiome among three ethnic groups. The central point represents the root of the tree (bacteria), and each ring represents the next lower taxonomic level (phylum to species).

patients and healthy individuals. Analysis of within-sample species richness showed that there were no significant differences in the α- and β-diversities between the groups (Figure S1).

In order to further explore the differences in the composition of gut microbiome, we used Humann3 to quantify the relative abundance, and the results showed that at phylum level, Bacteroidetes (47.7%), Firmicutes (39.8%), Actinobacteria (8.5%) and Proteobacteria (3.4%) were most abundant across the three ethnic groups (Figure 3A). At the genus level, *Prevotella*, *Bacterioides*, *Roseburia* and *Bifidobacterium* were most abundant (Figure 3B). Interestingly, we discovered that compositional profiles differed between the sarcopenia patients and healthy individuals. LEfSe analysis identified several different bacteria in each group. At the genus/species level, the relative abundances of *Bacteroides vulgatus*, *Bifidobacterium*, *Roseburia* and *Faecalibacterium prausnitzii* were higher in sarcopenia patients. On the other hand, *Eubacterium retale* (LDA = 3.71, *P*-value = 0.04) and *P. copri* (LDA = 4.42, *P*-value = 0.01) were higher in healthy individuals (Figure 3C). The cladogram showed that *P. copri* and its higher taxonomy taxa were more abundant in healthy individuals (Figure 3D). Considering the limitation of LEfSe[19] and to identify a more reliable bacterium that could be potentially used to produce beneficial effects, we used two-tailed unpaired Mann–Whitney *U* tests to determine whether the relative abundance of *E. retale* and *P. copri* were significantly different between sarcopenia patients and healthy individuals. We observed a significant reduction (20.77% vs. 15.03%, *P*-value = 0.01) in the average relative abundance of *P. copri* in the sarcopenia group compared with the healthy group (Figure 3E).

As age and gender are known factors influencing gut microbiome composition[20,21] and ethnic group also influence gut microbiome composition, we have performed a multivariate association analysis. After adjusting the factors of age, gender and ethnic group, the genus *Prevotella* is still significantly lower in sarcopenia patients compared with healthy individuals ($\beta = -8.81$, nominal *P*-value = 0.004). A different but non-significant trend was observed for the species *P. copri*; the lack of statistical significance ($\beta = -5.44$, nominal *P*-value = 0.058) may have been due to the limited sample size (Table S4).

We then investigated the potential contributions to biological function between the two groups. We performed KEGG annotation and enrichment analyses (Figure S2) of identified differential genes, the results showed that several amino acids biosynthesis and metabolism pathway were enriched, including biosynthesis of BCAAs, amino acid metabolism, metabolism of other amino acids (Figure 3F, Table S5).

In summary, we demonstrated that *Prevotella*, *P. copri* and the potential to produce BCAA were less abundant in sarcopenia patients across the three ethnic groups.

Branched chain amino acid fecal metabolite levels are lower in sarcopenia patients

Metabolites produced by gut microbiota are documented to influence the health and disease of the host. Because the functions of the differential gut microbiome are related to BCAA synthesis and metabolism, we investigated the relevance of BCAAs to sarcopenia by analysis of the metabolome. We investigated changes in the fecal metabolites using

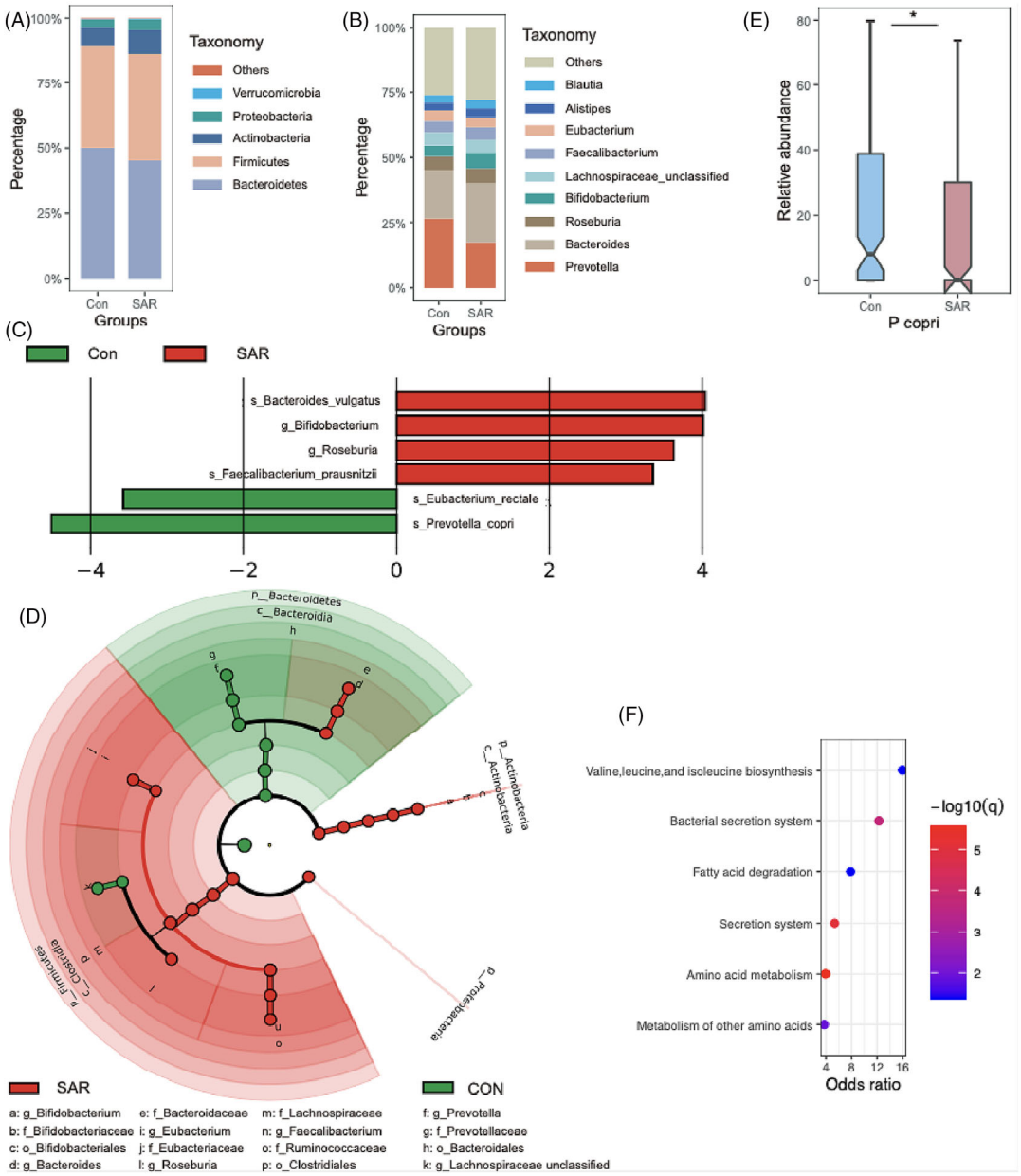

Figure 3 The relative abundance of *Prevotella copri* was higher in the healthy people compared with sarcopenia patients. (A, B) Phylum-level (A) and genus-level (B) taxonomic distribution of the gut microbiome among healthy older people and sarcopenia patients on average. (C) LDA scores of the differentially abundant taxa in gut microbiome from healthy people and sarcopenia patients (taxa with LDA score >2 and a significance of a < 0.05 are shown). (D) Cladogram, generated from LEfSe analysis, represented gut microbiome taxa enriched in healthy people and sarcopenia patients. The central point represents the root of the tree (bacteria), and each ring represents the next lower taxonomic level (phylum to genus). (E) Boxplot with notch showed the relative abundance of *P. copri*. Boxes represent the IQRs between the first and third quartiles, and the line inside the box represents the median. (F) Functional enrichment (KEGG) analysis showed the differential gene contact between healthy older people and SAR patients were enriched in valine, leucine, and isoleucine biosynthesis and fatty acid degradation pathways. *$P < 0.05$; **$P < 0.01$; Wilcoxon rank-sum test.

ultra-performance liquid chromatography.[22] To do a comprehensive search for differential metabolites, metabolites from the fecal samples of the 141 sarcopenia patients and 142 healthy individuals were evaluated using both positive and negative ion scan modes. In total, 1663 metabolites were identified and then allocated to molecular classes using the Human Metabolome Database (Table S1 and Table S2). To ensure the stability of the instrument and good reproducibility of samples, PCoA analysis was performed showing samples of fecal metabolites after quality control were aggregated with good reproducibility in the positive and negative ion modes, indicating that the testing system was stable

(Figure 4A,B). The metabolic patterns of the gut microbiota were evaluated using PLS-DA and orthogonal partial least squares-discriminant analysis (OPLS-DA) (Figure S3).

We found that 216 out of 1663 (12.99%) metabolites were significantly different between the sarcopenia and healthy groups. Specifically, the sarcopenia group showed significantly raised levels of 19 metabolites (Table S1) and significantly reduced levels of 197 metabolites (Table S2) (Figure 4C). Lipids and lipid-like molecules and organic acids and their derivatives were two majority metabolite classes reduced in sarcopenia patients (Figure 4D). In addition, specific reductions in the levels of several BCAAs and their metabolites observed in the sarcopenia group, including isoleucyl-isoleucine, isoleucylproline, valyl-valine, gamma-glutamylvaline, gamma-glutamylisoleucine and serylvaline (Figure 4E).

Taken together, pathway associated with BCAA biosynthesis were found to be enriched in the differential microbiota with BCAA levels were lower in sarcopenia patients, potentially originated from the gut microbiome.

Branched chain amino acid fecal levels were higher in the blood of mice gavaged with live Prevotella copri

As *P. copri* showed the greatest differences in abundance between sarcopenia patients and healthy individuals and pathways associated with BCAA biosynthesis were enriched (Figure 3F) with higher BCAA levels in healthy individuals (Figure 4E), we questioned whether *P. copri* can increase BCAA levels and thus relieve the symptoms of sarcopenia. Then a mouse model was then used to examine the possible protective role of *P. copri* in the gut and the muscle mass and function of the host.

We gavaged 20-month-old mice with LPC, heat-killed *P. copri*, BCAA and saline for 8 weeks (Figure 5A). To confirm the successful gavage of LPC, we assessed fecal samples of mice using qRT-PCR. The results showed there was a significant increase of absolute abundance of *P. copri* in the live bacteria group (Figure 5B) **in each cage**, indicating successful colonization in the intestinal tracts of the mice by *P. copri*. Then, we quantified the concentrations of BCAA in the plasma and found that BCAA levels in mice gavaged with LPC and BCAA group were higher than those gavaged with PBS (Figure 5C). These results indicated that increasing the abundance of *P. copri* in the gut might enhance the BCAA levels in the circulation.

Prevotella copri mitigated decreases in muscle function in old mice

As blood BCAA level was closely related with muscle function,[23] we were wondering what the influence of *P. copri*

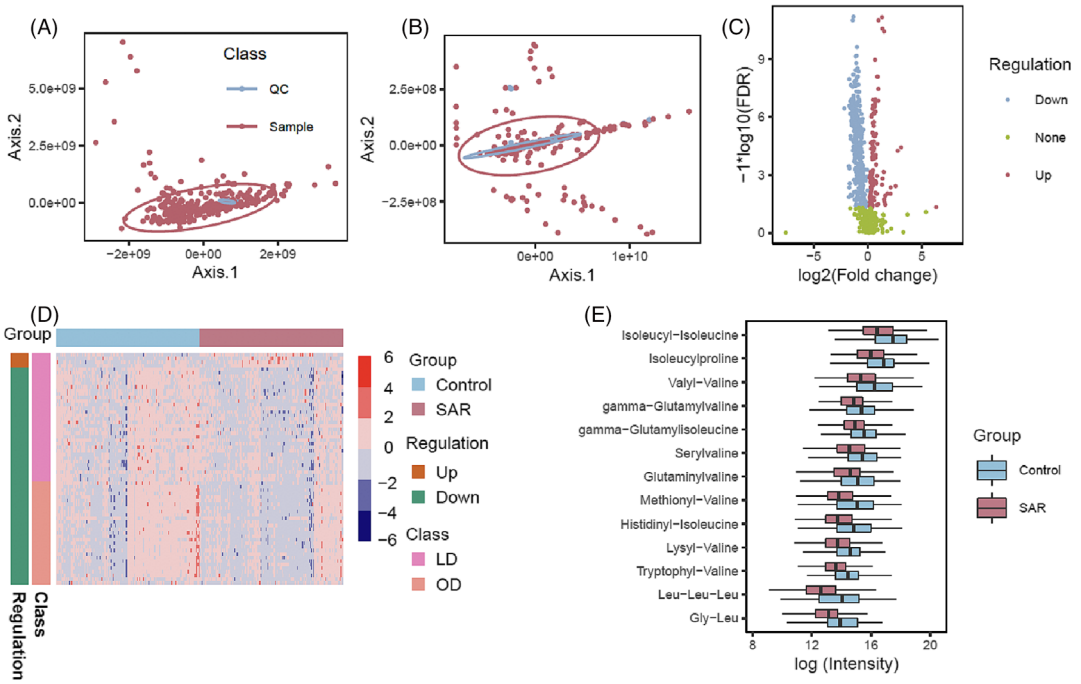

Figure 4 BCAAs were enriched in the healthy people. (A, B) PCoA analysis showed remarkable stability of the instrument and good reproducibility of samples in positive ion mode (A) and negative ion mode (B). (C) Volcano plot showed the differentially regulated metabolites in gut from healthy older people and SAR patients (VIP > 1; CV < 0.3; ratio > 1.5 or <1/1.5; Wilcox BH $P < 0.05$). (D) Heatmap showed the differentially regulated metabolites in two main classes. (LD: lipids and lipid-like molecules; OD: organic acids and derivatives). (E) Boxplot showed the differentially regulated branched-chain amino acids and their derivatives.

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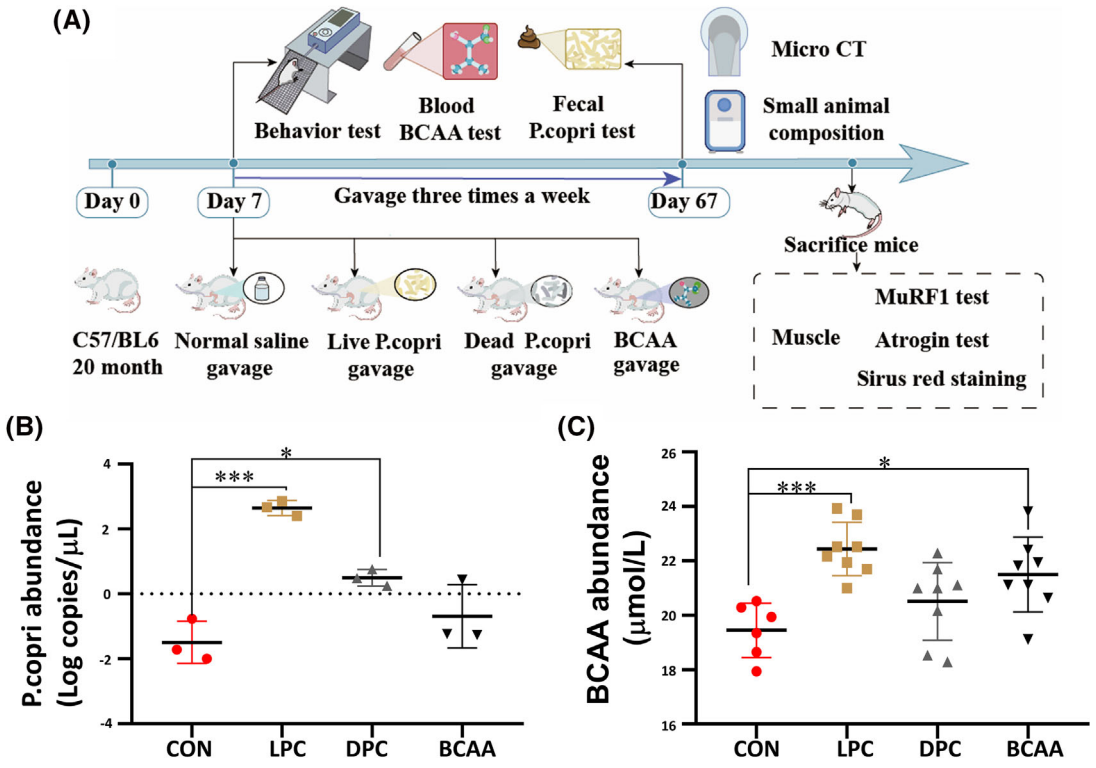

Figure 5 BCAA level was higher in old mice gavaged with live *P. copri*. (A) Schematic diagram of the mouse experiments. Live *P. copri* (LPC) was administrated by oral gavage to C57BL/6 mice three times per week at a dose of 5×10^8 cfu/0.1 mL PBS for 8 weeks. Gavage of sterile PBS or the same dose of heat-killed *P. copri* (DPC) were used as controls. Gavage of BCAA with the concentration of 1250 mg/kg. (B) The DNA absolute abundance of *P. copri* in the mice feces after the gavage experiment. The *y*-axis of the graph displayed the logarithm base 10 of *P. copri* DNA copy number in 1 μL. Each dot represented *P. copri* abundance of fecal in each cage, which was collected from all the mice cage-housed together. (C) The blood branched chain amino acid (BCAA) concentration level of the mice after the gavage experiment, N = 7–8. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; one way ANOVA test.

on muscle function in old mice. To ensure the consistency of muscle mass and function in the baseline, we obtained baseline measurements of weight, wire hanging time, number of rod turns, rod speed to fall, time on rotor, grid hanging time, forelimb grip strength and four limb grip strength, and no significant differences were observed (Figure S4).

After gavage intervention, it was found that the group of mouse gavaged with LPC had significantly longer wire hanging time (P = 0.0018), longer time on rotor (P = 0.0001), longer grid hanging time (P = 0.0169), larger forelimb grip strength ($P < 0.0001$) and larger limb grip strength ($P < 0.0001$), indicating gavage with LPC mitigated muscle function decline (Figure 6A–F).

In addition, we also conducted Sirius Red staining, for it can reflect the staining of collagen fibres in muscle. We observed a significant higher content in Collagen I (red) and Collagen III (green) in the soleus muscle (Figure S5a and Figure 5b) and rectus femoris in the group gavaged with LPC (Figure S5c,d). These results suggested improved muscle activity efficiency, improved the muscle contraction, and endurance.

All these results indicated gavaged with LPC mitigated muscle function decline, implying that it can delay sarcopenia.

Prevotella copri mitigated muscle mass loss in old mice

To explore the factors underlying the observed better muscle function in mice gavaged with LPC, we further assessed the muscle mass in mice. We conducted the body composition test and micro-computed tomography scan. It showed that the lean muscle mass percentage (P = 0.0157) and the leg area was higher (P = 0.0031) in the group gavaged with LPC (Figure 7C,D). While the fat mass percentage showed no difference among the four groups (Figure S6f). Besides, we found the weight of rectus femoris (P = 0.0168) and gastrocnemius muscle (P = 0.0335) was greater than the control group after dissection (Figure 7A,B). All the results showed that LPC treatment attenuated muscle mass loss in old mice.

To investigate the underlining mechanisms of LPC treatment mitigated muscle mass loss in old mice, we tested the expression of muscle protein synthesis and degradation genes in skeletal muscle. As Atrogin and MURF 1 are both important regulators of ubiquitin-mediated protein degradation in skeletal muscle, they are key markers of muscle loss and disuse muscle atrophy.[24] To determine the impact of LPC on

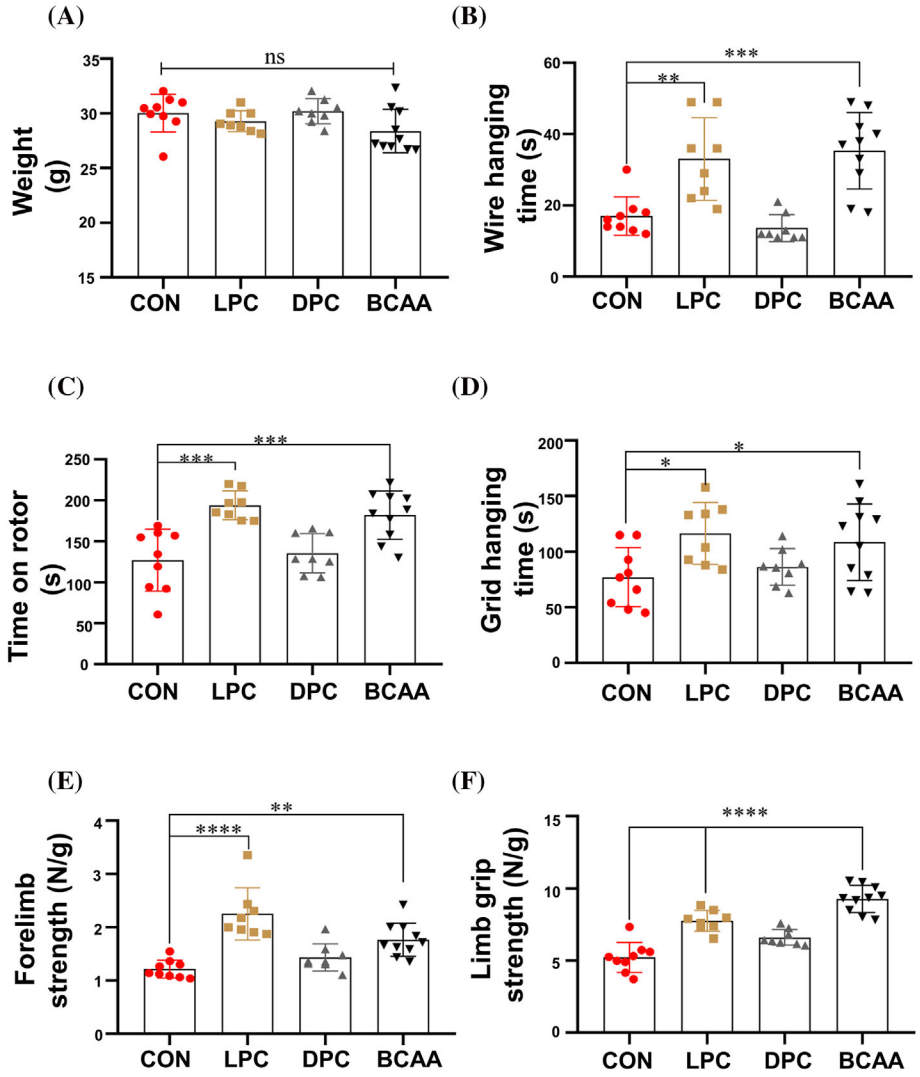

Figure 6 Muscle function was improved in old mice gavaged with live *P. copri*. (A) There was no difference of the body weight among the four groups. (B) The wire hanging time was longer in the group of LPC and BCAA compared with the control group. (C) The time on the rotor was longer in the group of LPC and BCAA compared with the control group. (D) The grid hanging time was longer in the group of LPC and BCAA compared with the control group. (E, F) The forelimb grip strength and four limb grip strength adjusted by weight were stronger in the group of LPC and BCAA compared with the control group. N = 8–10, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. One way ANOVA test.

protein degradation, we quantified the expression of these protein degradation genes using qRT-PCR. Our results indicated lower expression of MURF 1 (P = 0.0104) and a non-significant decrease in Atrogin (P = 0.0603) in the skeletal muscle tissue of mice gavaged with LPC (Figure 7E,F). Corresponding to it, we also investigated the expression of muscle protein synthesis genes such as MCK, MHC-1 and MHC-2B, but there was no significant difference observed among the four groups (Figure S6a–c).

Taken together, LPC treatment significantly mitigated muscle function and mass decline, indicating that *P. copri* may play therapeutic potential role to attenuate sarcopenia.

Discussion

This study demonstrated and verified changes in the human gut microbiome and metabolome associated with sarcopenia using an integrated multi-omic framework in a multi-ethnic cross-sectional study. Many differences in the microbiome compositions between individuals with sarcopenia and those without were observed. Specifically, the abundance of *Bifidobacterium* was higher in the sarcopenia group while that of *Prevotella* was higher in healthy individuals. In addition, BCAA levels were reduced in the sarcopenia group. Gavage with LPC protected mice against muscle loss and

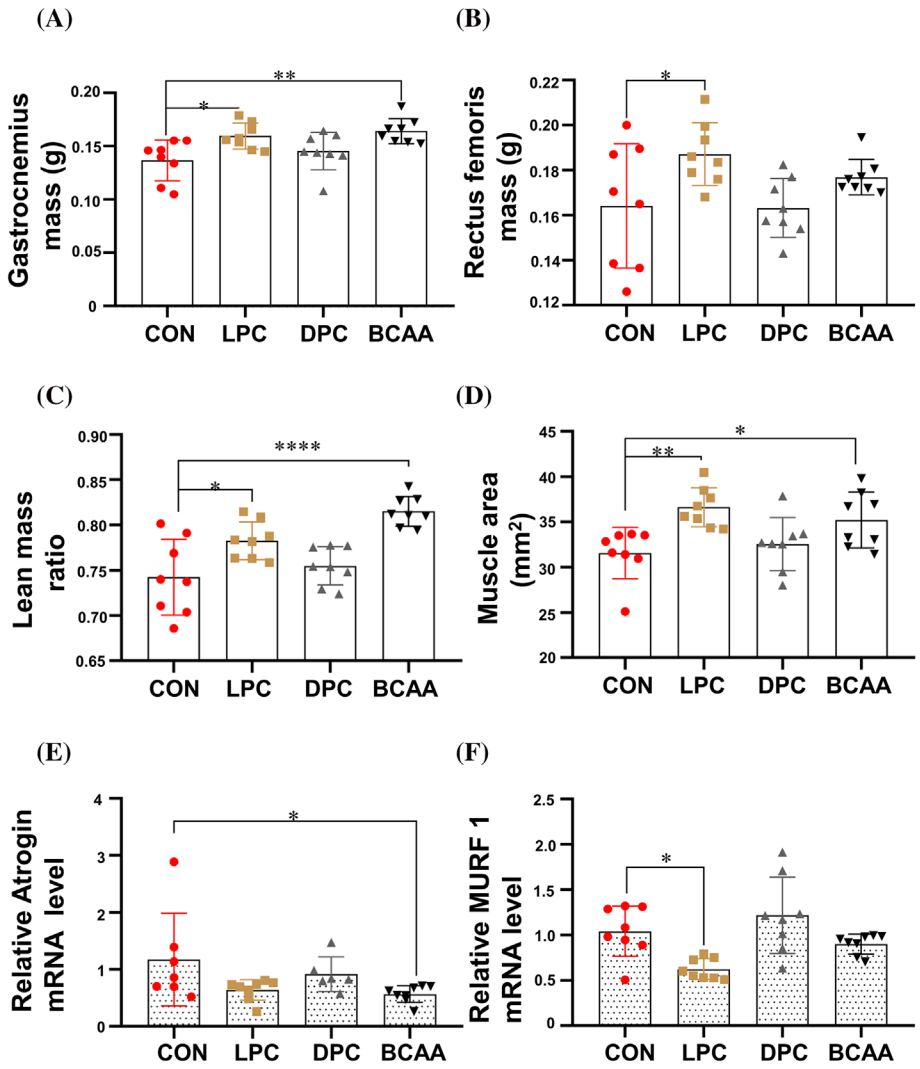

Figure 7 Muscle mass was greater in old mice gavaged with live *P. copri.* (A) The weight of gastrocnemius was higher in the group of LPC and BCAA after dissection. (B) The weight of rectus femoris was higher in the group of LPC and BCAA after dissection. (C) Lean mass ratio was higher in the group of LPC and BCAA tested by small animal body composition analyser. (D) The largest muscle area of the mice leg was greater in the group of LPC and BCAA measured by micro-computed tomography. (E) The group gavaged with BCAA had lower relative mRNA expression of Atrogin compared with the control group. (F) The group gavaged with live *P. copri* had lower relative mRNA expression of MURF 1 compared with the control group. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (A–F: one way ANOVA test; G–H: Wilcoxon rank sum test).

enhanced muscle function. These findings suggested that there is an association between the microbiome and sarcopenia and that targeting the gut microbiota might be an effective way to treat sarcopenia.

There is accumulating evidence of a relationship between the gut microbiota and sarcopenia. A recently published (1 October 2022) paper showed that the abundance of *Prevotella* and *P. copri* was significantly lower (*P*-value = 0.021 and *P*-value = 0.018, respectively) in 27 older adults with sarcopenia and 33 without sarcopenia from large-independent cohorts of European individuals (both male and female).[25] These results are consistent with our findings that the abundance of *Prevotella* and *P. copri* were higher in healthy individuals. However, some studies discovered different results. For example, a study in Xiangya Hospital included 1417 participants with 10% prevalence of sarcopenia, and they found that sarcopenia patients had higher relative abundance of *Desulfovibrio piger*, *Clostridium symbiosum*, *Hungatella effluvii*, *Bacteroides fluxus*, *Absiella innocuum*, *Coprobacter secundus* and *Clostridium citroniae* than healthy individuals.[26] In addition, researchers from Peking Union Medical College Hospital observed an overall reduction in microbial diversity in sarcopenia. And the genera *Lachnospira*, *Fusicantenibacter*, *Roseburia*, *Eubacterium* and *Lachnoclostridium* were significantly less abundant in sarcopenia.[27] The BIOSPHERE study from Italy found a reduced abundance of *Eubacterium* in frail elderly patients while that of *Bifidobacterium* was increased.[28] In addition, another study observed higher levels

of *Bifidobacterium longum* in sarcopenia patients compared with the healthy individuals, although the difference was not statistically significant.[29]

In addition, the mechanism of the gut-muscle-axis was investigated in mouse model in some studies. In a study on aged mice, researchers found that an altered *Sutterella* to *Barneseilla* ratio was linked to skeletal muscle function.[30] In addition, *Lactobacillus casei Shirota* supplementation was found to attenuate age-related sarcopenia in SAMP8 mice[31] while Bindels et al. reported that oral supplementation with *Lactobacillus* species decreased the symptoms of sarcopenia in a mouse model of acute leukaemia.[32] While according to a recent meta-analysis about gut microbiota and sarcopenia, *Bacteroides fragilis* gnotobiotic mice showed higher function and muscle mass compared with germ-free mice. Besides, *Lactobacillus* and *Bifidobacterium* strains restored age-related muscle loss. Other studies reported seven probiotic supplements *Saccharomyces boulardii* (SB), *Lactobacillus casei* LC122 (LC122), *Bifidobacterium longum* BL986 (BL986), *Lactobacillus paracasei* PS23 (LPPS23), *Lactobacillus salivarius* SA-03 (SA-03), *Lactobacillus plantarum* TWK10 (LP10) and *Bifidobacterium longum* OLP-01 (OLP-01) were beneficial to muscle growth and function in animal models.[33] To clearly study the effect of gut microbiome on sarcopenia, more studies are required to clarify the mechanism underlying the regulation of the gut-muscle axis in animal models.

The detailed knowledge of *P. copri*, which was found to be significantly decreased in the sarcopenia group in our study, is relatively limited due to both difficulties in culturing and its non-pathogenicity.[34] It is a common human gut bacterium in non-westernized populations, and its role in health and disease remains controversial. Fielding et al. tested the gut microbiota of elderly individuals with differing physical abilities twice with a one-month interval using the short physical performance battery test and found that there was a steady increase in *Prevotella* in the microbiomes of highly active elderly individuals.[35] After transplanting the gut microbiota of the two groups of elderly patients into mice, the authors found an increased abundance of Prevotellaceae and *Prevotella* in the intestines of mice transplanted with feces from the physically active group, with increases in grip strength of 6.4%.[36] However, some studies found significant reductions in *Prevotella* have also been found in the feces of frail older adults.[37,38] These contradictory results may be due to the huge strain difference of *P. copri* with distinct gene repertoires. Besides, distinct genetic and functional traits of human intestinal *P. copri* strains were associated with different habitual diets.[39]

Animal studies have found that feeding prebiotics can increase the abundance of Prevotellaceae and *Prevotella* in the gut of obese mice, along with increased muscle mass and decreased fat mass.[40] Chen et al. observed a positive association between *P. copri* abundance in the microbiome and fat accumulation in pigs fed on a formula diet as well as with the concentrations of obesity-related metabolites in serum such as lipopolysaccharides, aromatic amino acids, BCAA and arachidonic acid metabolites.[36] These authors also reported activation of chronic inflammation mediated by TLR4 and mTOR signalling after *P. copri* colonization in germ-free mice, suggesting that *P. copri* could upregulate the expression of genes associated with lipogenesis and fat deposition.[36] In our research, we found that *P. copri* gavage in mice could increase the muscle mass and function with decreased expression of protein degradation genes. However, the exact mechanism between *P. copri* and muscle mass needs more research.

Importantly, our findings suggested the potential of treating sarcopenia patients through regulation of the gut microbiota. Further investigations into the molecular mechanisms in which the gut microbiota and BCAA influence muscle mass and function were required.

Limitations

This study also had some limitations. First, our metagenomic and metabolomic analyses were carried out specifically in western China. Therefore, our results may not necessarily be generalized to other populations. Secondly, the population was small with only 283 samples, more samples was needed to do validation. But we did animal studies to verify the mechanism of the differential bacteria we found. Thirdly, we did not adjust dietary habits of the different ethnic groups as it was difficult to quantify.

Conclusions

Using metagenomic and metabolomic analyses, our study showed that the level of *P. copri* and BCAA were lower in sarcopenia patients across three ethnic groups in WCHAT study. Gavaged with LPC in mice can diminish muscle mass loss function decline and further attenuate sarcopenia. Our study demonstrates previously unknown associations between the gut microbiota, circulating branched-chain amino acids, and sarcopenia, suggesting the possibility of targeting the gut microbiota for sarcopenia treatment.

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Conflict of interest

None declared.

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Data availability statement

All the data that support the findings of this study are available from the corresponding author upon reasonable request. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics $ Bioinformatics 2021[42]) in National Genomics Data Center (Nucleic Acids Res 2022[43]), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA003953) that are publicly accessible at https://ngdc.cncb.ac.cn/search/?dbId=hra&q=HRA003953&page=1. The accession number was subPRO020223 and the project number was PRJCA013598. All code is available at https://github.com/JiqiuWu/SAR_2023.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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