Sarcopenia-related gut microbial changes are associated with the risk of complications in people with cirrhosis

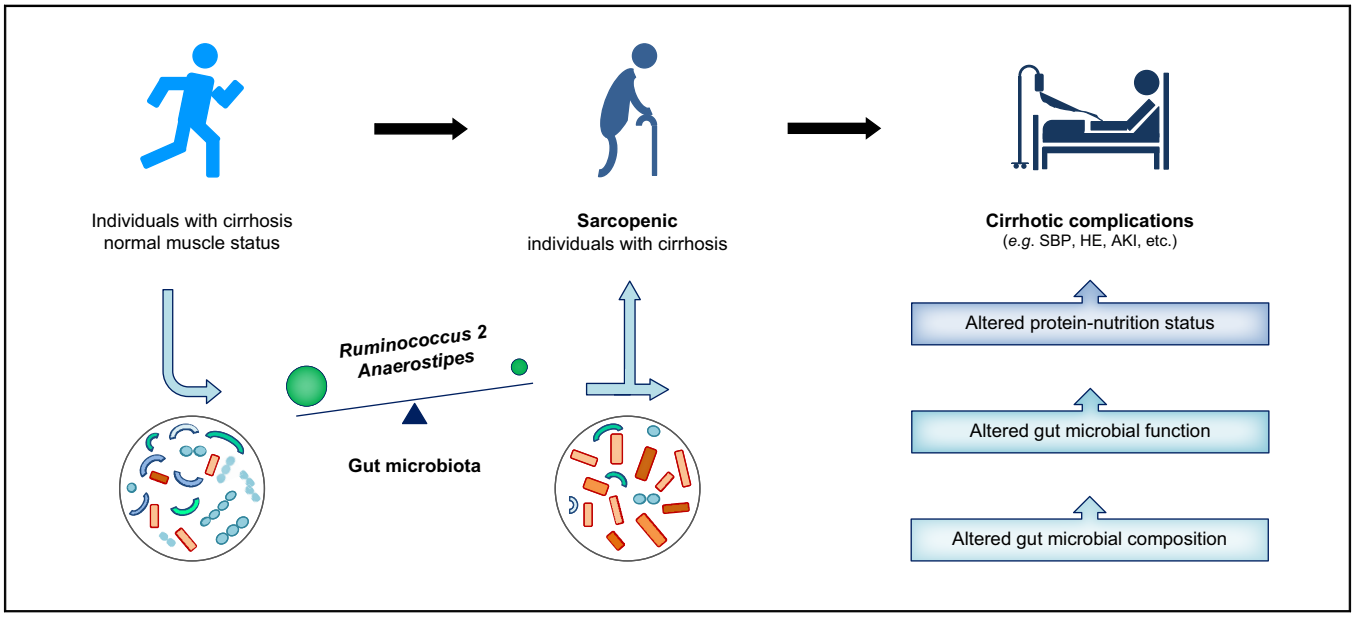
Authors

Pei-Chang Lee, **Kuei-Chuan Lee**, Tsung-Chieh Yang, Hsiao-Sheng Lu, Tsung-Yi Cheng, Yu-Jen Chen, Jen-Jie Chiou, Chi-Wei Huang, Ueng-Cheng Yang, Elise Chia-Hui Tan, Shih-Hsuan Chou, Yu-Lun Kuo, Bernd Schnabl, Yi-Hsiang Huang, Ming-Chih Hou

Correspondence

mchou@vghtpe.gov.tw (M.-C. Hou).

Graphical abstract



Highlights

- The composition and biosynthetic functions of gut microbiota are significantly changed in those with sarcopenia and cirrhosis.
- Individuals with cirrhosis and sarcopenia-related depletion of fecal *Ruminococcus 2* and *Anaerostipes* had more complications.
- Modifying gut microbiota may improve the clinical outcomes of individuals with cirrhosis and sarcopenia.

Impact and implications

The composition and biosynthetic functions of gut microbiota are significantly changed in individuals with sarcopenic cirrhosis. Those with a sarcopenia-related poor microbial signature, in which *Ruminococcus 2* and *Anaerostipes* were both depleted, had significantly more infectious and non-infectious complications, as well as more hospitalizations. These findings highlight the therapeutic potential of modifying the gut microbiota of individuals with sarcopenic cirrhosis to improve their clinical outcomes.

https://doi.org/10.1016/j.jhepr.2022.100619

Research article

JHEP|Reports

Sarcopenia-related gut microbial changes are associated with the risk of complications in people with cirrhosis

Pei-Chang Lee,[1,2,#] **Kuei-Chuan Lee**,[1,2,#] Tsung-Chieh Yang,[1,2] Hsiao-Sheng Lu,[1,2] Tsung-Yi Cheng,[1,2] Yu-Jen Chen,[1,2] Jen-Jie Chiou,[7] Chi-Wei Huang,[7] Ueng-Cheng Yang,[7] Elise Chia-Hui Tan,[5] Shih-Hsuan Chou,[3] Yu-Lun Kuo,[3] Bernd Schnabl,[4] Yi-Hsiang Huang,[1,2,6] Ming-Chih Hou[1,2,*]

[1]*Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan;* [2]*School of Medicine, College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan;* [3]*Biotools, Co, Ltd, New Taipei City, Taiwan;* [4]*Department of Medicine, University of California San Diego, La Jolla, CA, USA;* [5]*Department of Health Service Administration, College of Public Health, China Medical University, Taichung, Taiwan;* [6]*Institute of Clinical Medicine; College of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan;* [7]*Institute of Biomedical Informatics, National Yang Ming Chiao Tung University, Taipei, Taiwan*

JHEP Reports **2023.** https://doi.org/10.1016/j.jhepr.2022.100619

Background & Aims: Sarcopenia and gut dysbiosis are common in individuals with cirrhosis. However, the association between sarcopenia and microbial alterations, and the subsequent impact on cirrhotic outcomes are poorly understood. This study aimed to identify muscle-dependent microbial changes and related risks of cirrhotic complications.
Methods: From September 2018 to December 2020, 89 individuals with cirrhosis and 16 healthy volunteers were prospectively enrolled. Muscle and nutritional status, serum amino acids, and fecal microbiota were analyzed. The association between microbial signatures of sarcopenia and cirrhotic complications was investigated.
Results: A decline in muscle mass and strength were associated with gut microbial alterations in individuals with cirrhosis. The greatest microbial dissimilarity was observed between those with sarcopenia (both decline in muscle mass and strength) and those with normal-muscle status (p = 0.035). Individuals with sarcopenia had lower serum levels of alanine, valine, leucine, isoleucine, proline, tryptophan and ornithine. Besides, gut microbial functions associated with amino acid biosynthesis were significantly reduced in individuals with sarcopenia and cirrhosis. Depletion of *Dialister, Ruminococcus 2*, and *Anaerostipes* were associated with cirrhotic sarcopenia, and significantly correlated with the serum levels of amino acids. Individuals with coexistent depletion of *Ruminococcus 2* and *Anaerostipes* developed more infectious (44.4% *vs.* 3.0%) and non-infectious (74.1% *vs.* 3.0%) complications, and more hospitalizations (54 *vs.* 3) than those with cirrhosis with good microbial signatures (all p <0.001). In contrast, fecal enrichment of *Ruminococcus 2* and *Anaerostipes* independently decreased the risk of 1-year complications.
Conclusions: Sarcopenia-related fecal microbial alterations are associated with cirrhotic complications. These findings may facilitate measures to improve the outcomes of individuals with cirrhosis and sarcopenia by modifying gut microbiota.
Impact and implications: The composition and biosynthetic functions of gut microbiota are significantly changed in individuals with sarcopenic cirrhosis. Those with a sarcopenia-related poor microbial signature, in which *Ruminococcus 2* and *Anaerostipes* were both depleted, had significantly more infectious and non-infectious complications, as well as more hospitalizations. These findings highlight the therapeutic potential of modifying the gut microbiota of individuals with sarcopenic cirrhosis to improve their clinical outcomes.

Introduction

Sarcopenia is a common problem in individuals with cirrhosis, occurring at a prevalence rate of around 30–70%.[1,2] It has been reported that individuals with sarcopenia are at a higher risk of complications and mortality.[3–6] However, most studies focused only on muscle mass but ignored muscle strength and performance, which are determinant factors of sarcopenia.[4–7] Given that sarcopenia has important impacts on cirrhotic outcomes, identification of risk factors that could be therapeutically modulated may have significant clinical significance in cirrhosis.

Gut microbiota are well known to be associated with metabolism and to play a substantial role in host nutrition.[8,9] Altered microbial composition and function not only correlate with cirrhotic malnutrition, but also contribute to and worsen cirrhotic complications.[9–11] Besides, gut microbiota may influence muscle physiology by altering amino acid bioavailability, disturbing metabolites, and modulating pro-inflammatory cytokines.[12] According to recent studies, alterations of gut microbiota are observed in individuals with cirrhosis and sarcopenia.[7,13] However, it is unclear

Keywords: Sarcopenia; Cirrhotic complications; Microbiota; *Ruminococcus 2*; *Anaerostipes*.
Received 2 May 2022; received in revised form 29 September 2022; accepted 7 October 2022; available online 29 October 2022
Pei-Chang Lee and Kuei-Chuan Lee contribute equally to this study.
* Corresponding author. Address: Division of Gastroenterology and Hepatology, Department of Medicine, Taipei Veterans General Hospital, 201 Shih-Pai Road, Sec. 2, Taipei 11217, Taiwan; Tel.: 886-2-28757506, fax: 886-2-28739318.
E-mail address: mchou@vghtpe.gov.tw (M.-C. Hou).

whether these microbial alterations are dependent on muscle mass or muscle strength. Whether muscle-dependent microbiomes affect cirrhotic complications remains undetermined. Moreover, the role of these bacterial functions in cirrhosis is poorly understood. In this study, we aimed to investigate the impact of sarcopenia-dependent microbial alterations on cirrhotic complications. In addition, we analyzed alterations of gut microbiota according to the different muscular conditions of individuals with cirrhosis, microbial functional changes (focusing on the biosynthesis of amino acids), and correlations between microbiota and serum amino acids.

Materials and methods

Patients

From September 2018 to December 2020, 89 individuals with cirrhosis who had regular follow-up appointments or received medical treatment in Taipei Veterans General Hospital were prospectively enrolled in this study after informed consent. The diagnosis of cirrhosis was made according to the ultrasonography, computed tomography, or magnetic resonance imaging together with impaired liver function, or by liver biopsy.[14] In order to investigate fecal microbiota, patients who were prescribed lactulose, proton pump inhibitors, non-steroidal anti-inflammatory drugs, antibiotics, probiotics or prebiotics within 1 month were excluded from this study. The detailed inclusion and exclusion criteria for patient selection are presented in the supplementary materials and methods. In addition, 16 healthy volunteers who did not have any underlying medical disease and visited for health examination were also prospectively enrolled as controls. This study was approved by the Institutional Review Board of Taipei Veterans General Hospital (IRB numbers: 2017-09-013C and 2018-07-018C).

Definitions

According to the Asian Working Group for Sarcopenia 2019 consensus,[15] sarcopenia was defined as both a decline of muscle strength (handgrip strength <28 kg for men and <18 kg for women, measured using a digital hand dynamometer [TKK 5401 Grip-D; Takei Scientific Instruments Co., LTD., Tokyo, Japan]) and reduced muscle mass (appendicular skeletal muscle index <7.0 kg/m^2 in men and <5.4 kg/m^2 in woman, measured by dual-energy X-ray absorptiometry [Hologic Horizon A scanner, Hologic, Inc., Bedford, MA, USA] with APEX system software [version 5.6.0.5][16]). The 6-minute walk distance test was used to assess physical performance.[17] On the other hand, the diagnosis and management of cirrhotic complications were made according to the relevant EASL Clinical Practice Guidelines.[18]

Assessment of nutritional status and physical activity

All enrollees were examined for nutritional status by anthropometric measurements, including body weight, body mass index, mid-upper arm circumference, and tricuspid skinfold thickness as well as conventional serum laboratory data. Malnutrition screening and dietary status were assessed concomitantly upon enrollment by subjective global assessment (SGA), mini-nutritional assessment (MNA), and the malnutrition universal screening tool (MUST).[19] The level of physical activity and sitting time were assessed using the validated Korean version of the international physical activity questionnaire short form which enables the calculation of metabolic equivalent tasks (minutes per week).[20]

Measurements of serum amino acids

Serum samples were collected and stored at −80 °C until analysis. The serum was de-proteinized by adding the same volume of 10% sulfosalicylic acid containing an internal standard (norvaline 200 μM) and centrifuged at 12,000 g for 10 min at 25–28 °C. Derivatization of the supernatant was initiated by adding 20 μl of 10 mM 6-aminoquinoly-N-hydroxysuccinimidyl carbamate in acetonitrile. After incubation for 10 min, the reactant was mixed with an equal volume of Eluent A (20 mM ammonium formate, 0.6% formic acid, and 1% acetonitrile) and analyzed by Waters ACQUITY UPLC System (Waters Corp., Milford, MA, USA).[21] The details are presented in the supplementary materials and methods.

Processing and analysis of stool bacterial genomic data

Microbial genomic DNAs were extracted by using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). 12.5 ng of DNA from each specimen was used for the PCR. The hypervariable V3–V4 regions of bacterial 16S ribosomal RNA genes were amplified by PCR using the primers 341F V3 Illumina (5'-CCTACGGGNGGCWGCAG-3') and 806R V4 Illumina (5'- GACTACHVGGGTATCTAATCC-3').[22] The PCR product was purified by using AMPure XP beads. Next-generation sequencing was performed by the Illumina MiSeq Desktop Sequencer; and the ZymoBIOMICS™ Microbial Community DNA Standard (catalogue no. D6305, Zymo Research Corp., Irvine, CA, USA) was used as an internal control.

The 16S rRNA gene sequencing raw reads were processed and denoised using QIIME2 version 2020.11 and DADA2 plugin,[23,24] and were annotated with taxonomic classifications based on the Silva 132 database. Alpha diversity indices and principal coordinate analysis of microbiota were calculated and compared by Kruskal-Wallis test and permutational multivariate analysis of variance test with correction for multiple testing using the Benjamini-Hochberg procedure. Linear discriminant analysis with effect size was performed to determine the candidate taxa most likely to explain the differences between groups. Spearman's rank correlation analysis was performed for the microbial taxa and metabolites. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 2 (PICRUSt2) analysis was applied to predict the microbial functions and pathway inferences.[25] The details are described in the supplementary materials and methods.

Statistical analysis

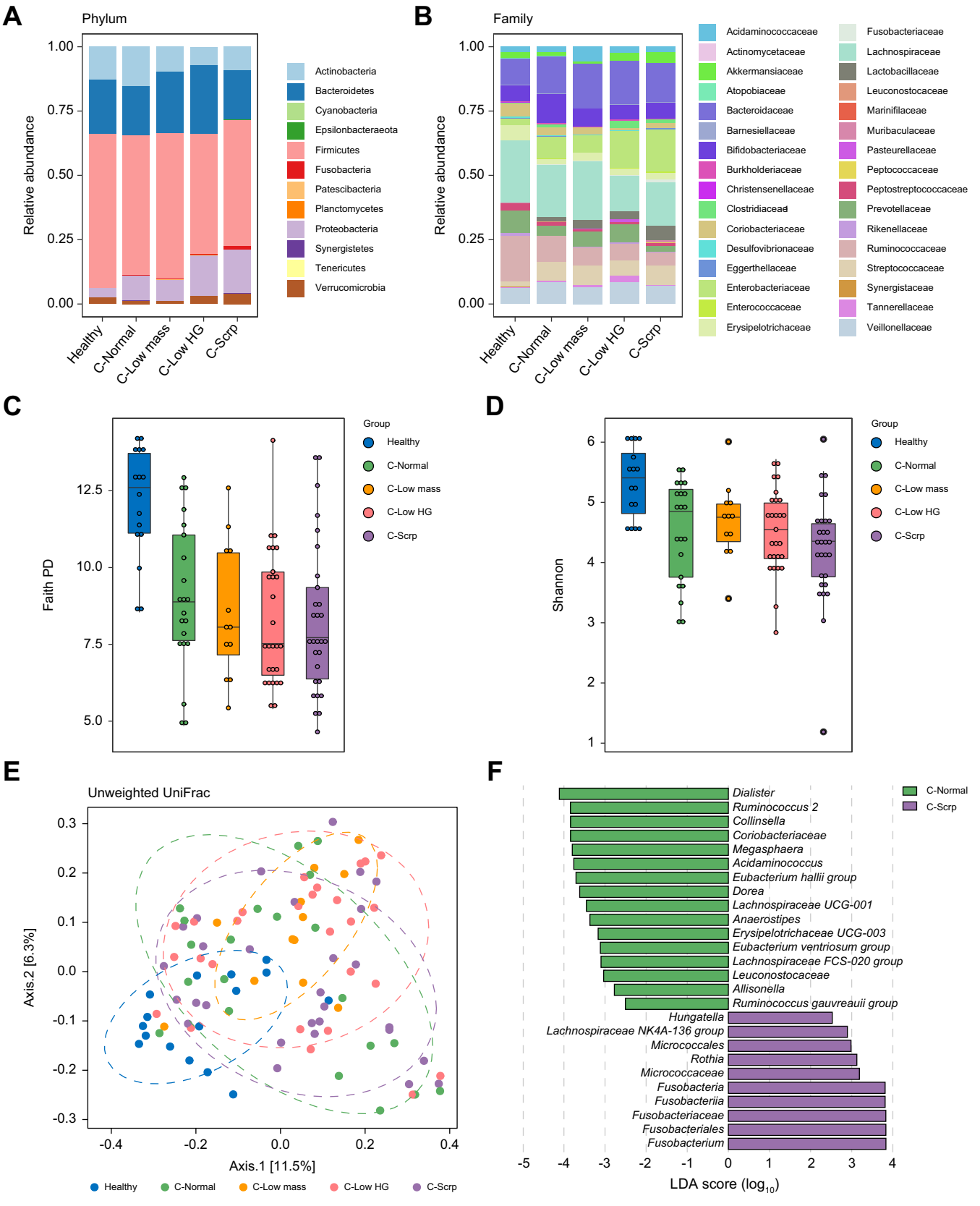
Continuous variables were expressed as median (interquartile range) and were compared by Mann-Whitney *U* test or Kruskal-Wallis test with a Bonferroni correction, while categorical variables were presented as frequencies (percentages) and were compared by Pearson's Chi-squared analysis or Fisher's exact test. The optimal cut-off values of microbial abundance to predict cirrhotic complications were assessed using the AUROC. The value with the highest Youden's index was considered as the optimal cut-off.[26] To identify the most important factors that were associated with cirrhotic sarcopenia and 1-year complications, we first performed a univariate logistic regression analysis to assess the association between each variable and the outcome of interest. Variables with statistical significance (p <0.2) and clinical relevance were considered as candidates for multivariate logistic regression. We also conducted LASSO logistic regression analysis to confirm the most useful prognostic risk factors for cirrhotic sarcopenia and 1-year complications. R software version 4.2.1 and the "glmnet" package (R Foundation for

Table 1. Baseline characteristics of healthy controls and individuals with cirrhosis according to muscle status.

	Healthy controls	Individuals with cirrhosis				
	Normal muscle status	Normal muscle status	Decreased muscle mass	Decreased muscle strength	Sarcopenia	Cirrhosis sarcopenia *vs.* normal muscle status *p* value
Characteristics	**n = 16**	**n = 21**	**n = 12**	**n = 27**	**n = 29**	
Age	58.4 (49.6–64.5)	58.8 (50.9–63.6)	53.9 (44.8–64.0)	65.7 (58.9–70.4)	62.7 (54.3–66.5)	0.234
Sex, male	13 (81.3)	17 (81.0)	9 (75.0)	18 (66.7)	25 (86.2)	0.706
Chronic hepatitis B	0 (0)	13 (61.9)	6 (50.0)	12 (44.4)	15 (51.7)	0.474
Chronic hepatitis C	0 (0)	4 (19.0)	1 (8.3)	8 (29.6)	1 (3.4)	0.148
Alcohol consumption	0 (0)	5 (23.8)	4 (33.3)	1 (3.7)	7 (24.1)	0.979
Body weight, kg	65.0 (59.9–69.5)	75.0 (67.8–85.0)	62.5 (54.4–70.0)	72.2 (64.1–81.0)	61.0 (55.5–67.7)	< 0.001
BMI, kg/m^2	23.3 (22.3–24.7)	27.5 (25.1–29.4)	21.0 (20.2–23.0)	27.6 (23.9–30.5)	22.4 (21.1–23.6)	< 0.001
MUAC, cm	26.5 (25.0–27.8)	31.0 (25.1–29.4)	24.0 (24.0–26.7)	29.0 (25.0–31.3)	24.5 (23.5–27.0)	< 0.001
Tricuspid skin fold, mm	20.0 (12.3–27.8)	24.0 (19.0–30.3)	22.0 (15.5–23.5)	22.5 (15.0–28.3)	14.0 (10.3–18.8)	0.002
MNA score	28.3 (27.5–28.9)	27.0 (25.0–28.0)	26.5 (25.5–27.5)	25.5 (24.0–27.0)	25.0 (23.5–26.5)	0.005
MNA score ≤23.5, n (%)	0 (0)	1 (4.8)	0 (0)	6 (22.2)	7 (24.1)	0.117
SGA score	0 (0–0)	1 (0–2)	1 (0–3)	3 (1–4)	3 (1–5)	0.004
SGA score >1, n (%)	0 (0)	7 (33.4)	3 (25.0)	20 (74.1)	21 (72.4)	0.006
Handgrip strength						
Male, kg	35.4 (31.6–40.0)	30.8 (29.7–33.4)	30.2 (28.6–32.7)	22.7 (20.4–24.1)	21.2 (16.5–24.8)	< 0.001
Female, kg	24.5 (22.6–31.8)	21.6 (18.9–28.7)	18.2 (18.0–44.0)	12.1 (8.6–15.1)	10.3 (7.6–12.6)	0.021
DEXA						
ASMI, kg/m^2 (male)	7.53 (7.17–7.94)	8.03 (7.57–8.75)	6.71 (6.18–6.83)	7.97 (7.34–8.63)	6.15 (5.63–6.78)	< 0.001
ASMI, kg/m^2 (female)	5.48 (3.06–5.83)	6.40 (6.29–6.54)	5.05 (4.28–5.21)	6.03 (5.60–7.99)	4.57 (4.28–4.95)	0.034
SMI, kg/m^2 (male)	17.50 (16.70–18.30)	19.30 (18.35–20.20)	16.10 (15.40–17.45)	19.50 (18.68–20.65)	15.90 (14.60–16.80)	< 0.001
SMI, kg/m^2 (female)	12.80 (12.10–13.80)	16.39 (15.50–17.30)	12.90 (11.20–13.70)	15.40 (14.45–20.40)	13.60 (12.63–14.05)	0.034
Fat mass, kg (male)	16.39 (14.79–20.00)	24.56 (21.40–27.07)	15.09 (13.68–18.90)	20.09 (15.94–26.48)	15.67 (13.25–17.62)	< 0.001
Fat mass, kg (female)	15.29 (14.46–20.83)	28.11 (21.20–33.74)	19.32 (18.23–19.62)	25.16 (18.64–32.31)	18.68 (10.22–25.96)	0.157
Fat-free mass, kg (male)	47.98 (46.09–51.11)	54.46 (51.18–60.05)	47.01 (43.42–51.16)	52.74 (49.20–55.59)	43.56 (40.43–47.62)	< 0.001
Fat-free mass, kg (female)	32.01 (31.84–35.35)	41.00 (35.83–47.23)	32.15 (27.36–33.11)	41.60 (34.67–48.37)	31.69 (29.85–38.94)	0.157
6MWD, meters	512.7 (455.8–569.7)	467.3 (455.8–498.5)	455.8 (444.3–512.7)	398.8 (299.1–512.7)	341.8 (227.9–398.8)	0.010
Presence of ascites, n (%)	0 (0)	5 (23.8)	4 (33.3)	13 (48.1)	19 (65.5)	0.004
Presence of varices, n (%)	0 (0)	12 (57.1)	6 (50.0)	16 (59.3)	14 (48.3)	0.536
Child-Pugh score	5 (5–5)	5 (5–7)	6 (5–7)	7 (5–9)	7 (6–9)	0.003
Child-Pugh class A/B/C	16/0/0 (100.0/0/0)	14/7/0 (66.7/33.3/0)	7/4/1 (58.3/33.3 8.3)	12/13/2 (44.4/48.1/7.4)	13/12/4 (44.8/41.4/13.8)	0.124
Laboratory data						
Albumin, g/dl	4.7 (4.5–4.7)	3.8 (3.3–4.2)	3.8 (3.3–4.6)	3.5 (2.7–3.8)	3.5 (3.0–3.9)	0.078
Total bilirubin, mg/dl	0.55 (0.47–0.67)	1.52 (1.09–2.08)	1.50 (0.94–3.51)	1.46 (0.97–2.17)	1.88 (1.14–3.47)	0.163
ALT, U/L	18 (15–27)	30 (24–39)	26 (22–47)	28 (23–37)	27 (17–41)	0.360
AST, U/L	22 (18–25)	42 (26–66)	40 (30–72)	33 (27–50)	35 (27–68)	0.775
Creatinine, mg/dl	0.85 (0.80–0.93)	0.85 (0.76–0.94)	0.90 (0.75–1.12)	0.97 (0.73–1.15)	0.82 (0.74–1.11)	0.992
Sodium, mmol/L	140 (138–141)	141 (137–142)	140 (135–141)	139 (136–142)	139 (136–141)	0.268
Leucocyte, /μl	5,200 (4,425–6,450)	4,600 (3,700–5,050)	4,050 (3,125–5,350)	4,000 (3,000–5,500)	4,600 (3,015–6,350)	0.753
Hemoglobin, g/dl	14.9 (13.8–15.9)	12.3 (10.2–14.7)	12.6 (10.7–14.0)	11.3 (9.5–13.1)	11.3 (10.4–13.2)	0.783
Platelet, K/μl	230 (195–259)	85 (59–126)	96 (49–153)	71 (42–104)	88 (47–123)	0.687
PT, INR	1.03 (0.97–1.08)	1.24 (1.15–1.35)	1.20 (1.03–1.34)	1.36 (1.24–1.45)	1.30 (1.16–1.48)	0.208

Continuous variables were expressed as median (interquartile ranges) and were compared by Mann-Whitney *U* test. Categorical variables were presented as frequencies (percentages) and were compared by Pearson's Chi-squared test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; (A)SMI (appendicular) skeletal muscle index; DEXA, dual-energy X-ray absorptiometry; INR, international ratio; MNA, mini-nutritional assessment; MUAC, mid upper arm circumference; MUST, malnutrition universal screening tool;PT, prothrombin time, SGA, subjective global assessment; 6MWD, 6-min walking distance.


A
Phylum
Relative abundance
1.00
0.75
0.50
0.25
0.00
Healthy
C-Normal
C-Low mass
C-Low HG
C-Scrp
Actinobacteria
Bacteroidetes
Cyanobacteria
Epsilonbacteraeota
Firmicutes
Fusobacteria
Patescibacteria
Planctomycetes
Proteobacteria
Synergistetes
Tenericutes
Verrucomicrobia
B
Family
Relative abundance
Acidaminococcaceae
Actinomycetaceae
Akkermansiaceae
Atopobiaceae
Bacteroidaceae
Barnesiellaceae
Bifidobacteriaceae
Burkholderiaceae
Christensenellaceae
Clostridiaceae
Coriobacteriaceae
Desulfovibrionaceae
Eggerthellaceae
Enterobacteriaceae
Enterococcaceae
Erysipelotrichaceae
Fusobacteriaceae
Lachnospiraceae
Lactobacillaceae
Leuconostocaceae
Marinifilaceae
Muribaculaceae
Pasteurellaceae
Peptococcaceae
Peptostreptococcaceae
Prevotellaceae
Rikenellaceae
Ruminococcaceae
Streptococcaceae
Synergistaceae
Tannerellaceae
Veillonellaceae
C
Faith PD
12.5
10.0
7.5
5.0
Group
Healthy
C-Normal
C-Low mass
C-Low HG
C-Scrp
D
Shannon
6
5
4
3
2
1
Group
Healthy
C-Normal
C-Low mass
C-Low HG
C-Scrp
E
Unweighted UniFrac
Axis.2 [6.3%]
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.2
0.0
0.2
0.4
Axis.1 [11.5%]
Healthy
C-Normal
C-Low mass
C-Low HG
C-Scrp
F
C-Normal
C-Scrp
Dialister
Ruminococcus 2
Collinsella
Coriobacteriaceae
Megasphaera
Acidaminococcus
Eubacterium hallii group
Dorea
Lachnospiraceae UCG-001
Anaerostipes
Erysipelotrichaceae UCG-003
Eubacterium ventriosum group
Lachnospiraceae FCS-020 group
Leuconostocaceae
Allisonella
Ruminococcus gauvreauii group
Hungatella
Lachnospiraceae NK4A-136 group
Micrococcales
Rothia
Micrococcaceae
Fusobacteria
Fusobacteriia
Fusobacteriaceae
Fusobacteriales
Fusobacterium
-5
-4
-3
-2
-1
0
1
2
3
4
LDA score (log10)

Statistical Computing, Vienna, Austria) were used to perform the Lasso logistic regression analysis. Other statistical analyses were performed using Statistical Package for Social Sciences (SPSS 26.0 for Windows, SPSS Inc, Chicago, IL) and GraphPad Prism 9 (GraphPad Software, San Diego, CA). For all analyses, p <0.05 was considered statistically significant.

Results

Baseline characteristics of patients

Among participants with cirrhosis, 21 (23.6%) had normal muscle mass and strength, 12 (13.5%) had reduced muscle mass (muscle wasting), 27 (30.3%) had reduced muscle strength, and 29 (32.6%) were classified as having sarcopenia, with both reductions of muscle mass and strength. More than half of participants with sarcopenia (55.2%) or reduced strength (55.5%) were classified at Child-Pugh class B or C. In contrast, most participants who maintained normal muscle strength were within Child-Pugh class A (Table 1). Notably, 28.3% of participants at Child-Pugh class A still had sarcopenia, and the prevalence rate was not significantly lower than that of patients at Child-Pugh B or C (37.2%, p = 0.368). On the other hand, although the prevalence of sarcopenia was higher in participants with ascites (41.3%), 23.3% of participants without ascites still presented with sarcopenia, which should not be overlooked (p = 0.069).

Compared to participants with cirrhosis who maintained normal muscle status, those with sarcopenia had a significantly increased risk of malnutrition (assessed by SGA and MNA score). Participants with sarcopenia had significantly worse anthropometric measurements, handgrip strength, skeletal and appendicular skeletal muscle indices, total body fat mass (less significant in females), and physical performance examined by the 6-minute walk test. They were significantly less physically active and spent more time sitting (Table 1 and Table S1).

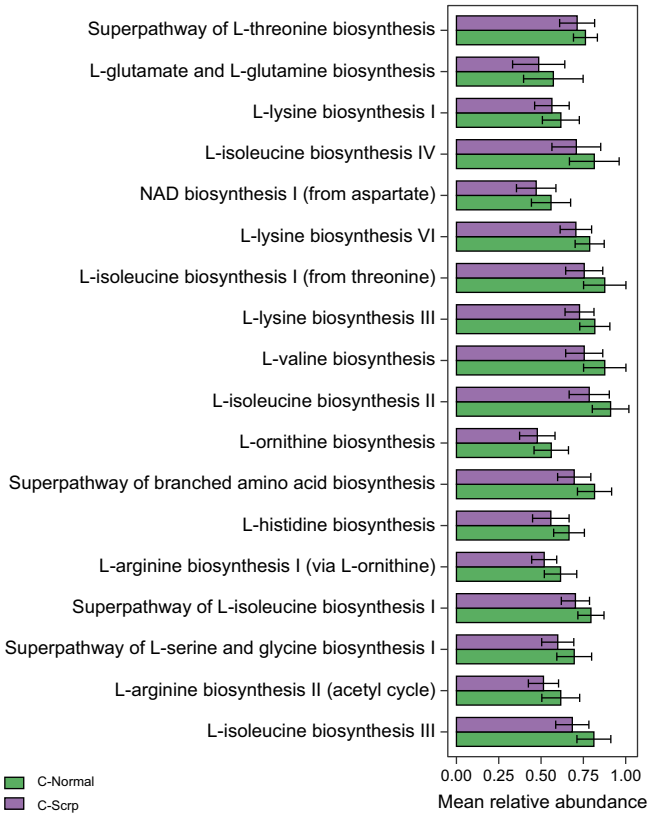


Fig. 2. The predicted metagenomic functional pathways of gut microbiota with significant differences between individuals with cirrhosis with different muscle status. All presented pathways are different with statistical significance (bars represent standard deviation; all p <0.05 by the Mann-Whitney U test). C-Normal, individuals with cirrhosis and normal-muscle status; C-Scrp, individuals with sarcopenic cirrhosis.

Gut microbiota changes with different muscle conditions of cirrhosis

Compared with healthy controls, the abundance of Proteobacteria was significantly increased in participants with cirrhosis, especially in those with sarcopenia (Fig. 1A). At the family level, apparent increases in Enterobacteriaceae and Streptococcaceae, as well as reduced Lachnospiraceae, Ruminococcaceae, and Prevotellaceae were observed in participants with cirrhosis, particularly in those with sarcopenia (Fig. 1B). The richness and evenness of microbiota measured by Faith's PD index and Shannon index were significantly decreased in participants with cirrhosis, regardless of their muscle status, compared to healthy controls (all p <0.05). However, no significant differences in alpha diversities were noted in individuals with cirrhosis with sarcopenia or normal-muscle status (Fig. 1C-D). In contrast, significant microbial dissimilarities measured by un-weighted UniFrac distances were observed not only between healthy controls and participants with cirrhosis (both p = 0.001 for healthy controls *vs.* cirrhosis+sarcopenia or cirrhosis+normal-muscle status), but also between those with cirrhosis with sarcopenia or normal-muscle status (p = 0.035) (Fig. 1E).

To determine the distinct microbiota associated with cirrhotic sarcopenia, the microbial profiles of participants with cirrhosis and sarcopenia or normal-muscle status were further investigated. According to linear discriminant analysis with effect size, a prominence of *Fusobacterium*, *Micrococcaceae* and *Rothia* (linear discriminant analysis score [$\log_{10}$] >3) was observed in participants

Fig. 1. The composition and diversity of gut microbiota in individuals with cirrhosis with different muscle conditions and healthy controls. Stacked bar plots of phylogenetic composition of common bacterial taxa (>0.1% abundance) at the (A) phylum and (B) family level in fecal samples of healthy controls, individuals with cirrhosis with normal muscle status (C-Normal), muscle mass depletion (C-Low mass), muscle strength weakness measured by handgrip (C-Low HG), and with sarcopenic cirrhosis (C-Scrp). Alpha diversity indices of gut microbiota measured by (C) Faith's PD index and (D) Shannon index (the boxes represent the interquartile range and the bold lines inside the boxes define the median; all p <0.001 for healthy controls vs. each group of individuals with cirrhosis, and p >0.05 between individuals with cirrhosis with different muscle status by the Kruskal-Wallis test, with correction for multiple comparisons by Benjamini-Hochberg procedure). (E) Principal coordinates analysis of gut microbiota measured by un-weighted Unifrac distance metrics (p = 0.001 for healthy *vs.* each group of individuals with cirrhosis; p = 0.035 for C-Scrp *vs.* C-Normal by permutational multivariate analysis of variance test with correction for multiple comparisons by Benjamini-Hochberg procedure). (F) LDA scores computed for differentially abundant taxa in the fecal microbiome. The length of each bar indicates the effect size associated with a taxon, which is significantly different when comparing to other groups (by the Kruskal-Wallis and Wilcoxon tests). HG, handgrip; LDA, linear discriminant analysis; Scrp, sarcopenia.

with cirrhosis and sarcopenia. In contrast, *Dialister, Ruminococcus 2, Collinsella, Coriobacteriaceae, Megasphaera, Acidaminococcus, Eubacterium hallii, Dorea, Lachnospiraceae*, and *Anaerostipes* were prominent in those with normal-muscle status (Fig. 1F). On the other hand, no significant microbial dissimilarities were observed according to the consumption of non-selective beta blockers, histamine-2 receptor antagonists, oral anti-diabetic drugs, statins or the underlying etiology of cirrhosis (Fig. S1).

Association of amino acids with gut microbiota and microbial functions

According to PICRUSt2 analysis, the microbial functions associated with the biosynthesis of several amino acids, including L-threonine, L-glutamine, L-glutamate, L-isoleucine, L-valine, L-ornithine, branched-chain amino acids (BCAAs) *etc.*, were significantly decreased in participants with cirrhosis and sarcopenia (p <0.001) (Fig. 2). Interestingly, the serum levels of BCAAs, including valine, leucine, and isoleucine, as well as alanine, proline, tryptophan and ornithine were significantly reduced in participants with cirrhosis and sarcopenia (Fig. 3 and Table S2). In addition, negative correlations were observed between serum proline, glutamine, threonine and gut microbes which were predominant in participants with cirrhosis and sarcopenia. In contrast, many amino acids, which are essential to muscle development, were positively correlated with bacteria that were predominant in participants with cirrhosis and normal-muscle status, including *Dialister, Anaerostipes*, *Lachnospiraceae, and Ruminococcus* (Fig. 4). Taken together, the correlation between sarcopenia-dependent microbiota and the reduction in serum amino acids may indicate an association with the decreased microbial biosynthesis of amino acids.

Distinct signature of microbiota associated with sarcopenia in cirrhosis

According to the ROC analyses, *Fusobacterium, Rothia, Dialister, Ruminococcus 2, Megasphaera, Anaerostipes,* and *Eubacterium Hallii* were probable discriminating microbiota to predict the risk of sarcopenia in cirrhosis (AUROC >0.6). Therefore, the abundances of these microbes and other clinical factors were subjected to LASSO logistic regression analyses. As presented in Table 2 and Fig. S2A-B, depleted fecal abundance of *Dialister*, *Ruminococcus 2* and *Anaerostipes* were selected as predictors of cirrhotic sarcopenia, with odds ratios of 6.823, 4.590, and 4.640, respectively. In addition, significant reduction in *Ruminococcus 2* and *Anaerostipes* were consistently noted in participants with Child-Pugh B or C cirrhosis (Fig. S3A-B).

Microbiota associated with sarcopenia determined the risk of 1-year cirrhotic complications

During the median follow-up period of 32.3 months (range: 15.1–35.8 months), 44 patients (49.4%) developed cirrhotic complications within the first year after enrollment. According to the LASSO regression analysis, a higher SGA score and presence of ascites were selected as predictors of 1-year cirrhotic complications. In contrast, hyperalbuminemia and fecal enrichment of genera *Ruminococcus 2* and *Anaerostipes* were independent protectors for 1-year cirrhotic complications with odds ratios of 0.151, 0.204, and 0.038, respectively (Table 3). On the other hand, the relative fecal abundances of *Ruminococcus 2* and *Anaerostipes* were not significantly different according to the presence of ascites, indicating their independent roles (Fig. S3C-D).

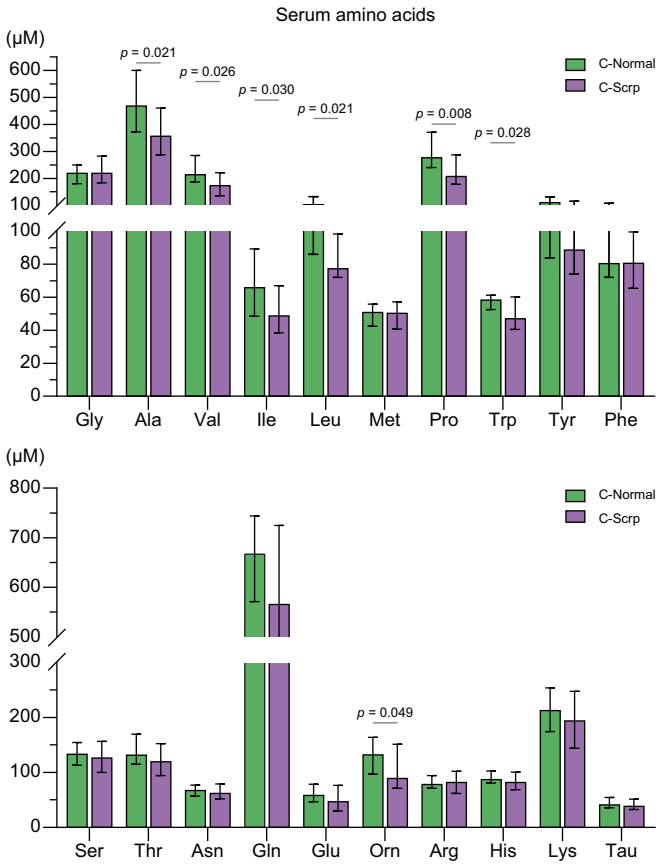


Fig. 3. Serum amino acids in individuals with cirrhosis with different muscle status. Serum levels of amino acids between C-Normal and C-Scrp. Statistical analysis was performed by the Mann-Whitney *U* test (The *p* values with statistical significance are presented in the plot). Ala, alanine; Arg, arginine; Asn, asparagine; C-Normal, individuals with cirrhosis and normal-muscle status; C-Scrp, individuals with sarcopenic cirrhosis; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Trp, tryptophan; Tyr, tyrosine; Thr, threonine; Val, valine.

Subgroup analyses according to the relative abundances of these two taxa are presented in Table 4. Participants with poor microbial signatures, in whom *Ruminococcus 2* and *Anaerostipes* were both depleted, developed more cirrhotic complications (both infectious and non-infectious events) and had more hospitalizations compared to those with good microbial signatures (p <0.001). These findings were consistent not only in participants with good liver reserves, but also among patients who were beyond Child-Pugh class A. Regarding specific complications, significantly more cases of hepatic encephalopathy (HE), acute kidney injury and spontaneous bacterial peritonitis were identified in participants with poor microbial signatures (p < 0.001; p = 0.002 and 0.022, respectively compared with their counterparts), especially in those who were at Child-Pugh class B or C. In participants with cirrhosis and ascites, the rates of HE, acute kidney injury, total complication events and the number of hospitalizations were also significantly higher in those with poor microbial signatures than in those with good signatures. The rate of spontaneous bacterial peritonitis was higher in participants with poor microbial signatures but without statistical significance (Table S3).

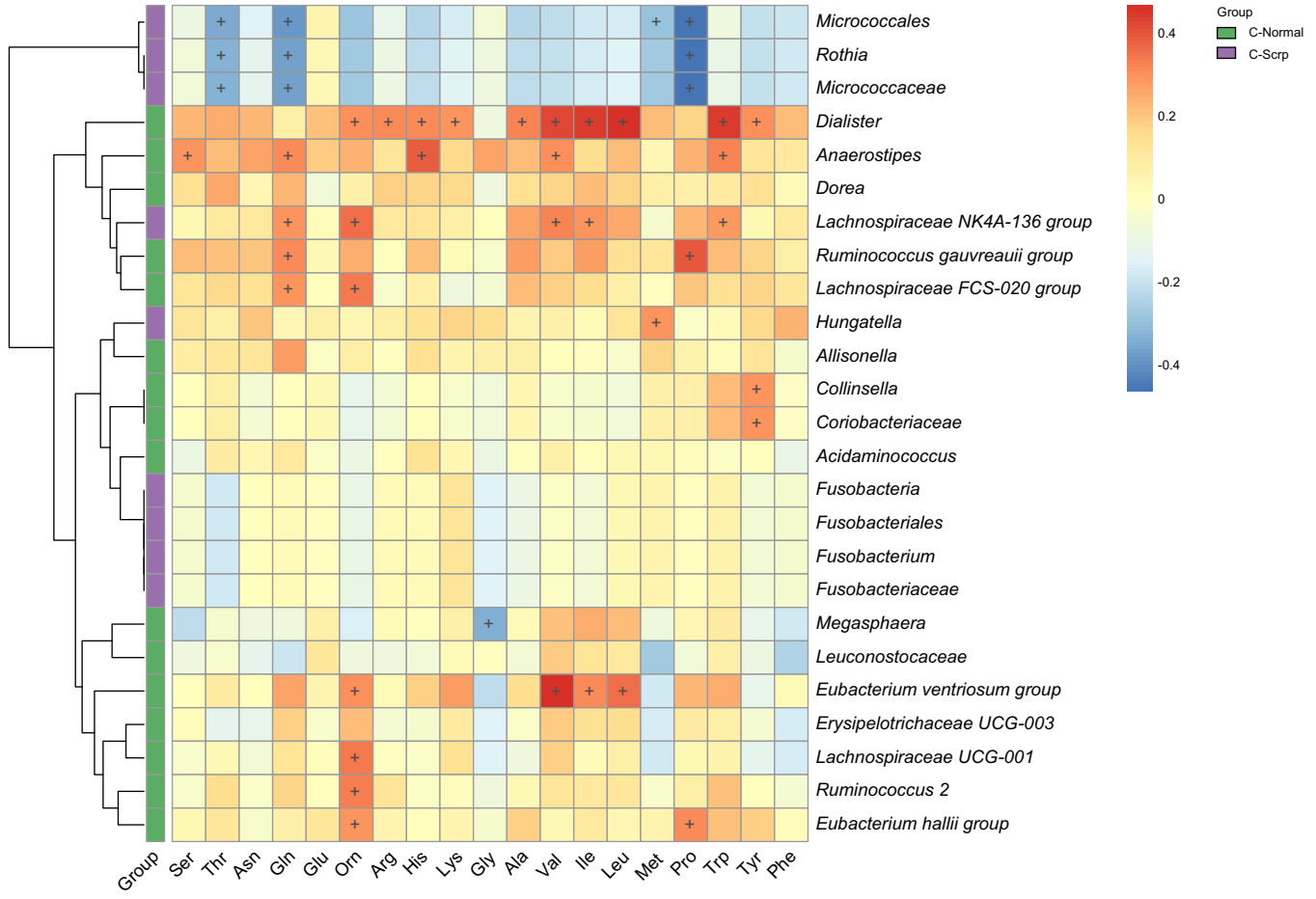


Fig. 4. Heat map of the correlation matrix between abundance of gut microbiota and the serum level of amino acids in individuals with cirrhosis. Red tones indicate positive correlations between the serum levels of amino acids and the abundance of differential intestinal microbes; blue tones indicate negative correlations. The plus sign indicates statistical significance (p <0.05 by the Spearman's rank correlation analysis). Ala, alanine; Arg, arginine; Asn, asparagine; C-Normal, individuals with cirrhosis and normal-muscle status; C-Scrp, individuals with sarcopenic cirrhosis; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Trp, tryptophan; Tyr, tyrosine; Thr, threonine; Val, valine.

Discussion

This is the first prospective study to demonstrate a significant association between sarcopenia-related gut microbial alterations and the development of complications in individuals with cirrhosis. Individuals with a sarcopenia-related poor microbial signature, in whom *Ruminococcus 2* and *Anaerostipes* were both depleted, had significantly more infectious and non-infectious complications as well as more 1-year hospitalizations. In addition, we observed a spectrum of microbial alterations from normal muscular status to muscle wasting, strength reduction and sarcopenia. We also found a significant reduction in microbial functions in the biosynthesis of numerous amino acids in individuals with cirrhosis and sarcopenia. Moreover, close correlations between serum levels of amino acids and sarcopenia-related gut microbiota were also discovered in individuals with cirrhosis.

An association between altered intestinal microbiota and age-related declines of muscle mass, composition, and function has been reported;[27] and the important role of the gut-muscle axis in the pathophysiology of sarcopenia has also been suggested.[28] In individuals with cirrhosis, a Chinese study reported that the Shannon index of gut microbiota was significantly decreased, and the abundance of *Escherichia coli* and *Peptostreptococcus stomatis* were increased in those with muscle wasting.[7] According to another Italian study, *Eggerthella* was found to be significantly enriched in those with sarcopenic cirrhosis; in contrast, *Methanobrevibacter, Prevotella* and *Akkermansia* were significantly reduced.[13] Additionally, a decrease in the abundance of *Dialister, Ruminococcus, Eubacterium, Dorea* and *Collinsella,* as well as an increase in *Rothia* were also noted in individuals with sarcopenic cirrhosis,[13] which are similar to the findings of our study. Although the gut microbiome is considered diverse among different ethnicities, and is critically influenced by environmental factors,[29] the microbial compositions in individuals with sarcopenic cirrhosis are similar between the Italian population and our cohort. In contrast, the effects of taking non-selective beta blockers, histamine-2 receptor antagonists, oral anti-diabetic drugs or statins on microbial composition were not significant in our patients. Besides, the etiology of cirrhosis did not cause obvious microbial dissimilarities. The unvaried causes of liver disease, mainly chronic

Table 2. Factors associated with sarcopenia in individuals with cirrhosis.

Characteristics	Case numbers	Univariate*			Multivariate*			LASSO#
		OR	95% CI	*p* value	OR	95% CI	*p* value	Coefficient
Age, y, >60 *vs.* ≤60	48/41	1.325	0.541–3.246	0.538				
Sex, male *vs.* female	69/20	2.273	0.684–7.550	0.180	5.617	1.233–25.577	0.026	
SGA score, >1 *vs.* ≤1	51/38	2.625	1.006–6.847	0.049			n.s.	
Etiology of cirrhosis, viral *vs.* non-viral	59/30	0.487	0.193–1.224	0.126			n.s.	
Alcohol consumption, yes *vs.* no	17/72	1.591	0.536–4.724	0.403				
NLR, >4.2 *vs.* ≤4.2	18/71	2.550	0.885–7.351	0.083			n.s.	
Platelet count, ≤100 *vs.* >100 K/μl	56/33	0.763	0.307–1.894	0.560				
Albumin, >3.5 *vs.* ≤3.5 g/dl	46/43	0.817	0.336–1.984	0.655				
Prothrombin time, INR >1.25 *vs.* ≤1.25	44/45	1.516	0.525–4.372	0.042			n.s.	
ALT, >40 *vs.* ≤40 U/L	19/70	1.697	0.597–4.822	0.321				
AST, >40 *vs.* ≤40 U/L	42/47	1.067	0.439–2.591	0.887				
Total bilirubin, >2.0 *vs.* ≤2.0 mg/dl	34/55	1.867	0.755–4.613	0.176			n.s.	
Presence of varices, yes *vs.* no	48/41	0.714	0.293–1.737	0.457				
Presence of ascites, yes *vs.* no	46/43	2.322	0.926–5.823	0.072			n.s.	
Fecal microbiota								
g *Fusobacterium,* enriched *vs.* depleted	33/56	1.625	0.656–4.027	0.294				
g *Rothia*, enriched *vs.* depleted	28/61	1.941	0.762–4.943	0.164			n.s.	
g *Dialister,* depleted *vs.* enriched	39/50	10.542	3.633–30.586	< 0.001	6.823	1.978–23.529	0.002	1.034
g *Ruminococcus 2,* depleted *vs.* enriched	49/40	9.375	2.895–30.360	< 0.001	4.590	1.148–18.357	0.031	0.630
g *Megasphaera,* depleted *vs.* enriched	57/32	2.931	1.043–8.239	0.041			n.s.	
g *Anaerostipes*, depleted *vs.* enriched	34/55	7.302	2.718–19.618	< 0.001	4.640	1.395–15.431	0.012	0.650
g *Eubacterium Hallii,* depleted *vs.* enriched	56/33	2.403	0.891–6.841	0.083			n.s.	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; g, genus; INR, international normalized ratio; NLR, neutrophil lymphocyte ratio; SGA, subjective global assessment.
LASSO logistic regression analysis.
* Binary logistic regression analysis.

hepatitis B, and the less potent effects of these drugs on microbiota may be responsible for these findings.

Interestingly in this study, we identified the important roles of the genera *Ruminococcus 2* and *Anaerostipes* not only in the presence of sarcopenia, but also in the development of complications and hospitalizations in individuals with cirrhosis. *Anaerostipes* is a butyrate-producing commensal which appears to be important for maintaining gut barrier function[30] and is positively associated with healthier muscle mass and function.[31] It is also reportedly less abundant in individuals with overt HE or alcohol-related liver disease.[32,33] On the other hand, *Ruminococcus* is found to be abundant in people with higher muscle mass but decreased in individuals with cirrhosis, particularly in those with HE.[10,34–36] Besides, the relative

Table 3. Risk factors associated with development of 1-year cirrhotic complications.

Characteristics	Case numbers	Univariate*			Multivariate*		LASSO#
		OR	95% CI	*p* value	OR	95% CI	Coefficient
Characteristics	48/41	1.815	0.781–4.219	0.166			
Sex	69/20	0.750	0.276–2.038	0.573			
Age, y, >60 *vs.* ≤60	51/38	4.500	1.818–11.138	0.001			0.437
Sex, male *vs.* female	59/30	0.645	0.266–1.564	0.332			
SGA score, >1 *vs.* ≤1	17/72	1.189	0.413–3.428	0.748			
Etiology of cirrhosis, viral *vs.* non-viral	18/71	2.437	0.823–7.219	0.108			
Alcohol consumption, yes *vs.* no	56/33	1.289	0.544–3.054	0.564			
NLR, >4.2 *vs.* ≤4.2	46/43	0.190	0.077–0.468	< 0.001	0.151	0.037–0.623	–0.638
Platelet count, ≤100 *vs.* >100 K/μl	44/45	2.250	0.840–6.028	0.107			
Albumin, >3.5 *vs.* ≤3.5 g/dl	19/70	0.900	0.326–2.484	0.839			
Prothrombin time, INR >1.25 *vs.* ≤1.25	42/47	1.800	0.776–4.175	0.171			
ALT, >40 *vs.* ≤40 U/L	34/55	1.845	0.776–4.388	0.166			
AST, >40 *vs.* ≤40 U/L	48/41	1.815	0.781–4.219	0.166			
Total bilirubin, >2.0 *vs.* ≤2.0 mg/dl	46/43	5.905	2.363–14.753	< 0.001			0.559
Presence of varices, yes *vs.* no							
Presence of ascites, yes *vs.* no	33/56	1.385	0.584–3.283	0.460			
Fecal microbiota	28/61	3.040	1.184–7.806	0.021			
g *Fusobacterium,* enriched *vs.* depleted	50/39	0.281	0.117–0.679	0.005			
g *Rothia*, enriched *vs.* depleted	40/49	0.090	0.033–0.246	<0.001	0.204	0.056–0.740	– 0.899
g *Dialister,* enriched *vs.* depleted	32/57	0.700	0.293–1.672	0.422			
g *Ruminococcus 2,* enriched *vs.* depleted	55/34	0.046	0.014–0.152	<0.001	0.038	0.008–0.175	– 0.208
g *Megasphaera,* enriched *vs.* depleted	33/56	0.348	0.142–0.856	0.021			

ALT, alanine aminotransferase; AST, aspartate aminotransferase; g, genus; INR, international normalized ratio; NLR, neutrophil lymphocyte ratio; SGA, subjective global assessment.
LASSO logistic regression model.
* Binary logistic regression analysis.

Table 4. Development of 1-year cirrhotic complications classified by sarcopenia-related gut microbial signatures.

	All cirrhosis				Child-Pugh class A				Child-Pugh class B or C			
	Microbial signature[#]				Microbial signature[#]				Microbial signature[#]			
	Good	Fair	Poor		Good	Fair	Poor		Good	Fair	Poor	
Cirrhotic complications	n = 33	n = 29	n = 27	*p* value	n = 25	n = 11	n = 10	*p* value	n = 8	n = 18	n = 17	*p* value
Number of patients with events, n (%)												
All complications	1 (3.0)	20 (69.0)*	23 (85.2)*	<0.001	0 (0)	7 (63.6)*	6 (60.0)*	< 0.001	1 (12.5)	13 (72.2)*	17 (100)*	<0.001
Infectious complications	1 (3.0)	13 (44.8)*	12 (44.4)*	<0.001	0 (0)	4 (36.4)*	0 (0)	0.001	1 (12.5)	9 (50.0)	12 (70.6)*	0.025
Non-infectious complications	1 (3.0)	18 (62.1)*	20 (74.1)*	<0.001	0 (0)	6 (54.5)*	6 (60.0)*	< 0.001	1 (12.5)	12 (66.7)*	14 (82.4)*	0.003
Specific complications												
Hepatic encephalopathy	0 (0)	6 (20.7)*	12 (44.4)*	<0.001	0 (0)	2 (18.2)	0 (0)	0.036	0 (0)	4 (22.2)	12 (70.6)*	0.001
Acute kidney injury	0 (0)	5 (17.2)*	9 (33.3)*	0.002	0 (0)	1 (9.1)	1 (10.0)	0.287	0 (0)	4 (22.2)	8 (47.1)*	0.039
Spontaneous bacterial peritonitis	0 (0)	4 (13.8)*	6 (22.2)*	0.022	0 (0)	1 (9.1)	0 (0)	0.197	0 (0)	3 (16.7)	6 (35.3)	0.054
Bacteremia	1 (3.0)	5 (17.2)	4 (14.8)	0.163	0 (0)	2 (18.2)	1 (10.0)	0.111	1 (12.5)	3 (16.7)	3 (17.6)	0.947
Total number of complication events	3	57[$]	54[$]	<0.001	0	14*	7*	< 0.001	3	43	47[$]	0.035
Total number of hospitalizations	3	47[$]	54[$]	<0.001	0	11*	7*	< 0.001	3	36	47[$]	0.032

* *p* <0.05 compared with patients presented good microbial signature, tested by Pearson's Chi-squared test.
[$] Kruskal-Wallis test with a Bonferroni correction.
[#] Gut microbial signatures: good signature, coexistent enrichment of *Ruminococcus 2* and *Anaerostipes;* poor signature, coexistent depletion of *Ruminococcus 2* and *Anaerostipes;* fair signature, only enrichment of *Ruminococcus 2* or *Anaerostipes*.

abundance of *Ruminoccocus* is negatively related to systemic inflammation or the model for end-stage liver disease score in individuals with cirrhosis.[36] As in this study, patients with coexisting depletion of these two microbial genera had significantly more events of HE, acute kidney injury and spontaneous bacterial peritonitis. These findings were consistent in patients within and beyond Child-Pugh class A, suggesting the independent role of sarcopenia-related microbiota on the outcomes of cirrhosis. HE could be improved by fecal microbiota transplant from slurries enriched with the microbiota that are deficient in individuals with HE.[37] Similarly, supplementation of specific microbiota discovered in this study may also be applied in clinical practice to improve sarcopenia and reduce complications in individuals with cirrhosis.

It has been suggested that gut microbiota and their metabolites regulate host muscle growth and function via many different pathways.[38] Microbes were reported to be capable of synthesizing amino acids and making them available to the host.[39] Besides, it could promote protein anabolism in the host by increasing bioavailability of amino acids and insulin responsiveness in the skeletal muscle.[40] According to an animal study, increased production of BCAAs by the intestinal microbiota is associated with improved protein synthesis by the host.[41] In addition, amino acids, particularly glutamine, glutamate, and aspartate, are major energy substrates for the intestinal mucosa that are associated with nutritional status.[42] According to PICRUSt2 analyses in this study, the microbial function in biosynthesis of numerous amino acids, including BCAAs, glutamine, glutamate, *etc*., was significantly reduced in individuals with sarcopenic cirrhosis. Besides, the decreased serum levels of amino acids in those with sarcopenic cirrhosis were closely correlated with alterations in the microbiota. Given these findings, decreased microbial activities in the biosynthesis of amino acids may have a putative impact on the anabolism of amino acids and the development of cirrhotic sarcopenia.

This study had some limitations. First, this was an observational study, and we could not determine the causal relationship between different bacteria and cirrhotic sarcopenia. Fecal amino acids were not analyzed because the amino acids from the undigested or unabsorbed dietary protein and amino acids from the desquamated epithelial cells of human gut would lead to the bias of examination.[43] As a substitute, we investigated the gut-muscle axis by means of PICRUSt 2 analysis (to predict the functional change in amino acid biosynthesis of gut microbiota) and correlation analyses (to investigate the association between microbial composition and serum amino acids of individuals with cirrhosis). Second, longitudinal data on microbial composition were lacking for stability assessment. However, we regularly followed our patients to ensure that their lifestyle habits were consistent during the follow-up period. Any medical need was well-documented as a complication. Third, cirrhosis in our patients mainly resulted from chronic hepatitis B. One has to be cautious when applying these results to other populations. Fourth, a validation study, either via another cohort or an animal experiment, is needed to verify our findings.

In conclusion, alterations in the gut microbial composition and functions in the biosynthesis of amino acids are associated with the development of sarcopenia and complications in individuals with cirrhosis. Individuals with sarcopenia-related poor microbial signatures had significantly worse clinical outcomes. These findings highlight a potential therapeutic strategy to improve the clinical outcomes of individuals with sarcopenic cirrhosis by modifying the gut microbiota.

Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; (A)SMI, (appendicular) skeletal muscle index; HE, hepatic encephalopathy; MNA, mini-nutritional assessment; MUST, malnutrition universal screening tool; OR, odds ratio; PICRUSt2, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States version 2; SGA, subjective global assessment.

Financial support

This study was supported by grants from the Ministry of Science and Technology of Taiwan (MOST 107-2314-B-075-043, MOST 108-2314-B-075-057, MOST 109-2314-B-075 -096, MOST 109-2314-B-075-020-MY3, MOST 110-2314-B-075-050-MY3) and Taipei Veterans General Hospital (V108B-021, V109B-021, V110B-024). This study was supported in part by services provided by NIH center P30 DK120515 (B.S.).

Conflict of interest

B.S. has been consulting for Ferring Research Institute, HOST Therabiomics, Intercept Pharmaceuticals, Mabwell Therapeutics, Patara Pharmaceuticals and Takeda. B.S.'s institution UC San Diego has received research support from Axial Biotherapeutics, BiomX, CymaBay Therapeutics, NGM Biopharmaceuticals, Prodigy Biotech and Synlogic Operating Company. B.S. is founder of Nterica Bio. UC San Diego has filed several patents with B.S. as inventor.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Study concept and design: PC Lee, KC Lee, MC Hou; Acquisition of data: KC Lee, PC Lee, TC Yang, HS Lu, TY Cheng, YJ Chen; Analysis and interpretation of data: PC Lee, KC Lee, JJ Chiou, CW Huang, UC Yang, E-CH Tan, SH Chou, YL Kuo, B Schnabl; Drafting the manuscript: PC Lee; Critical revision: KC Lee, B Schnabl, YH Huang, MC Hou; Statistical analysis: PC Lee, JJ Chiou, CW Huang, UC Yang, E-CH Tan; Obtained funding: MC Hou, PC Lee, B Schnabl; Study supervision: MC Hou, KC Lee.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgement

We highly appreciate Hiu-Yan Kong, Ying-Chun Xu, Kai-Ting Wang, Yi-Hsin Chang, and Kee-Deng Jou for their excellent technical assistance. We would like to acknowledge the support of the GenoInfo Core Facility (C1) funded by National Core Facility for Biopharmaceuticals (NCFPB) of Ministry of Science and Technology of Taiwan (MOST 110-2740-B-A49A-501) for providing the bioinformatics and microbiome data analysis.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100619.

References

[1] Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. J Hepatol 2016;65:1232–1244.
[2] Merli M, Berzigotti A, Zelber-Sagi S, Dasarathy S, Montagnese S, Genton L, et al. EASL Clinical Practice Guidelines on nutrition in chronic liver disease. J Hepatol 2019;70:172–193.
[3] Montano-Loza AJ, Meza-Junco J, Prado CM, Lieffers JR, Baracos VE, Bain VG, et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol 2012;10:166–173. 173.e161.
[4] Huguet A, Latournerie M, Debry PH, Jezequel C, Legros L, Rayar M, et al. The psoas muscle transversal diameter predicts mortality in patients with cirrhosis on a waiting list for liver transplantation: a retrospective cohort study. Nutrition 2018;51–52:73–79.
[5] Kang SH, Jeong WK, Baik SK, Cha SH, Kim MY. Impact of sarcopenia on prognostic value of cirrhosis: going beyond the hepatic venous pressure gradient and MELD score. J Cachexia Sarcopenia Muscle 2018;9:860–870.
[6] Praktiknjo M, Book M, Luetkens J, Pohlmann A, Meyer C, Thomas D, et al. Fat-free muscle mass in magnetic resonance imaging predicts acute-on-chronic liver failure and survival in decompensated cirrhosis. Hepatology 2018;67:1014–1026.
[7] Ren X, Hao S, Yang C, Yuan L, Zhou X, Zhao H, et al. Alterations of intestinal microbiota in liver cirrhosis with muscle wasting. Nutrition 2021;83:111081.
[8] Chu H, Duan Y, Yang L, Schnabl B. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. Gut 2019;68:359–370.
[9] Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology 2014;146:1513–1524.
[10] Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol 2014;60:940–947.
[11] Trebicka J, Macnaughtan J, Schnabl B, Shawcross DL, Bajaj JS. The microbiota in cirrhosis and its role in hepatic decompensation. J Hepatol 2021;75(Suppl 1):S67–S81.
[12] Bindels LB, Delzenne NM. Muscle wasting: the gut microbiota as a new therapeutic target? Int J Biochem Cel Biol 2013;45:2186–2190.
[13] Ponziani FR, Picca A, Marzetti E, Calvani R, Conta G, Del Chierico F, et al. Characterization of the gut-liver-muscle axis in cirrhotic patients with sarcopenia. Liver Int 2021;41:1320–1334.
[14] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749–1761.
[15] Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, et al. Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. J Am Med Dir Assoc 2020;21:300–307.e302.
[16] Belarmino G, Gonzalez MC, Sala P, Torrinhas RS, Andraus W, D'Albuquerque LAC, et al. Diagnosing sarcopenia in male patients with cirrhosis by dual-energy X-ray absorptiometry estimates of appendicular skeletal muscle mass. JPEN J Parenter Enteral Nutr 2018;42:24–36.
[17] Carey EJ, Steidley DE, Aqel BA, Byrne TJ, Mekeel KL, Rakela J, et al. Six-minute walk distance predicts mortality in liver transplant candidates. Liver Transpl 2010;16:1373–1378.
[18] Angeli P, Bernardi M, Villanueva C, Francoz C, Mookerjee RP, Trebicka J, et al. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol 2018;69:406–460.
[19] Tandon P, Raman M, Mourtzakis M, Merli M. A practical approach to nutritional screening and assessment in cirrhosis. Hepatology 2017;65:1044–1057.
[20] Bassett Jr DR. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1396.
[21] Tsai HI, Lo CJ, Zheng CW, Lee CW, Lee WC, Lin JR, et al. A lipidomics study reveals lipid signatures associated with early allograft dysfunction in living donor liver transplantation. J Clin Med 2018;8:30.
[22] Nossa CW, Oberdorf WE, Yang L, Aas JA, Paster BJ, Desantis TZ, et al. Design of 16S rRNA gene primers for 454 pyrosequencing of the human foregut microbiome. World J Gastroenterol 2010;16:4135–4144.
[23] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–583.
[24] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852–857.
[25] Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 2020;38:685–688.
[26] Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biom J 2005;47:458–472.
[27] Grosicki GJ, Fielding RA, Lustgarten MS. Gut microbiota contribute to age-related changes in skeletal muscle size, composition, and function: biological basis for a gut-muscle Axis. Calcif Tissue Int 2018;102:433–442.
[28] Ticinesi A, Lauretani F, Milani C, Nouvenne A, Tana C, Del Rio D, et al. Aging gut microbiota at the cross-road between nutrition, physical frailty, and sarcopenia: is there a gut-muscle Axis? Nutrients 2017;9:1303.
[29] Bajaj JS, Idilman R, Mabudian L, Hood M, Fagan A, Turan D, et al. Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. Hepatology 2018;68:234–247.
[30] Belzer C, Chia LW, Aalvink S, Chamlagain B, Piironen V, Knol J, et al. Microbial metabolic networks at the mucus layer lead to diet-independent butyrate and vitamin B(12) production by intestinal symbionts. mBio 2017;8. e00770-17.
[31] Ticinesi A, Nouvenne A, Cerundolo N, Catania P, Prati B, Tana C, et al. Gut microbiota, muscle mass and function in aging: a focus on physical frailty and sarcopenia. Nutrients 2019;11:1633.
[32] Bloom PP, Luévano Jr JM, Miller KJ, Chung RT. Deep stool microbiome analysis in cirrhosis reveals an association between short-chain fatty acids and hepatic encephalopathy. Ann Hepatol 2021;25:100333.
[33] Lang S, Fairfied B, Gao B, Duan Y, Zhang X, Fouts DE, et al. Changes in the fecal bacterial microbiota associated with disease severity in alcoholic hepatitis patients. Gut Microbes 2020;12:1785251.
[34] Liu C, Cheung WH, Li J, Chow SK, Yu J, Wong SH, et al. Understanding the gut microbiota and sarcopenia: a systematic review. J Cachexia Sarcopenia Muscle 2021;12:1393–1407.
[35] Chen Y, Yang F, Lu H, Wang B, Lei D, Wang Y, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 2011;54:562–572.
[36] Bajaj JS, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol 2012;302:G168–G175.

[37] Bajaj JS, Kassam Z, Fagan A, Gavis EA, Liu E, Cox IJ, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: a randomized clinical trial. Hepatology 2017;66:1727–1738.
[38] Lahiri S, Kim H, Garcia-Perez I, Reza MM, Martin KA, Kundu P, et al. The gut microbiota influences skeletal muscle mass and function in mice. Sci Transl Med 2019;11. eaan5662.
[39] Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr 2000;130:1857s–1864s.
[40] Beaumont M, Portune KJ, Steuer N, Lan A, Cerrudo V, Audebert M, et al. Quantity and source of dietary protein influence metabolite production by gut microbiota and rectal mucosa gene expression: a randomized, parallel, double-blind trial in overweight humans. Am J Clin Nutr 2017;106:1005–1019.
[41] Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 2014;10:723–736.
[42] Wu G. Functional amino acids in nutrition and health. Amino Acids 2013;45:407–411.
[43] Trommelen J, Tomé D, van Loon LJC. Gut amino acid absorption in humans: concepts and relevance for postprandial metabolism. Clinical Nutrition Open Science 2021;36:43–55.