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Characterization of the gut-liver-muscle axis in cirrhotic patients with sarcopenia

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Abstract

Background & Aim: Sarcopenia is frequent in cirrhosis and is associated with unfavourable outcomes. The role of the gut-liver-muscle axis in this setting has been poorly investigated. The aim of this study was to identify gut microbiota, metabolic and inflammatory signatures associated with sarcopenia in cirrhotic patients.

Methods: Fifty cirrhotic patients assessed for the presence of sarcopenia by the quantification of muscle mass and strength were compared with age- and sex-matched

Abbreviations: ALM, appendicular lean mass; ALM_{BMI}, appendicular lean mass to body mass index ratio; BCAAs, branched-chain amino acids; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CCL, C-C motif chemokine ligand; ChEBI, chemical entities of biological interest; CRP, C-reactive protein; CutC, choline trimethylamine-lyase; CXCL, C-X-C motif chemokine ligand; DMA, dimethylamine; DXA, dual X-ray absorptiometry; FDR, false discovery rate; FGF21, fibroblast growth factor 21; FMO3, flavin-containing monooxygenase 3; FNIH, Foundation for the National Institutes of Health; GMCSF, granulocyte-macrophage-colony-stimulating factor; HBV, hepatitis B virus infection; HCV, hepatitis C virus; HMDB, human metabolome database; IFN, interferon; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; log2FC, log2 fold change; LPS, lipopolysaccharide; LVs, latent variables; MetPA, metabolic pathway analysis; MPO, myeloperoxidase; MSEA, metabolite set enrichment analysis; NAFLD, nonalcoholic fatty liver disease; PCoA, principal coordinates analysis; PLSDA, partial least squares-discriminant analysis; rlog, regularized logarithm transformation; SCFAs, short chain fatty acids; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; TNF, tumour necrosis factor; VIP, variable importance in projection; ZO1, zonulin-1.

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A written informed consent was obtained from all participants prior to enrolment.

controls. A multiomic analysis, including gut microbiota composition and metabolomics, serum myokines and systemic and intestinal inflammatory mediators, was performed.

Results: The gut microbiota of sarcopenic cirrhotic patients was poor in bacteria associated with physical function (*Methanobrevibacter*, *Prevotella* and *Akkermansia*), and was enriched in *Eggerthella*, a gut microbial marker of frailty. The abundance of potentially pathogenic bacteria, such as *Klebsiella*, was also increased, to the detriment of autochthonous ones. Sarcopenia was associated with elevated serum levels of pro-inflammatory mediators and of fibroblast growth factor 21 (FGF21) in cirrhotic patients. Gut microbiota metabolic pathways involved in amino acid, protein and branched-chain amino acid metabolism were up-regulated, in addition to ethanol, trimethylamine and dimethylamine production. Correlation networks and clusters of variables associated with sarcopenia were identified, including one centred on *Klebsiella*/ethanol/FGF21/*Eggerthella*/*Prevotella*.

Conclusions: Alterations in the gut-liver-muscle axis are associated with sarcopenia in patients with cirrhosis. Detrimental but also compensatory functions are involved in this complex network.

KEYWORDS

cirrhosis, ethanol, gut microbiota, metabolomics, sarcopenia

Lay summary

- The role of the gut-liver-muscle axis in the development of sarcopenia in patients with cirrhosis is largely unknown.
- The gut microbiota contributes to sarcopenia through metabolic and pro-inflammatory networks, but, at the same time, it may exert compensatory functions to limit muscle wasting.
- Our findings can help to identify patients with cirrhosis who may benefit from personalized treatments to halt the progression of muscle wasting.

1 | INTRODUCTION

Muscle wasting and physical function impairment affect 30%-70% of patients with cirrhosis.[1] Several factors are invoked in the pathogenesis of muscle atrophy in this patient population. Reduced use of carbohydrates as a source of energy at the expense of proteins, hormonal changes, malnutrition, gastrointestinal dysfunction, increased resting energy expenditure and low physical activity are responsible of the loss of muscle mass in cirrhotic patients.[2]

Sarcopenia has been associated with poor quality of life and has been shown to independently predict negative outcomes, including reduced survival, in patients with cirrhosis.[3] Interestingly, while liver transplantation can cure cirrhosis, its effects on sarcopenia are variable with either improvement, stability or worsening being reported in literature.[3,4] Therefore, a better understanding of the pathophysiology of sarcopenia in this setting is needed to plan targeted therapeutic interventions.[5]

In older adults, sarcopenia has been associated with a state of chronic, low-grade inflammation, termed inflamm-ageing. This condition shares the same pathophysiological traits of meta-inflammation, which is typical of metabolic disorders and is associated with changes in the gut microbiota.[6] Cirrhosis is a paradigm of chronic inflammation driven by gut dysbiosis and increased intestinal permeability. However, whether alterations in gut microbiota are associated with sarcopenia in cirrhotic patients has yet to be established.

To fill this gap in knowledge, the present study aimed at characterizing the gut-liver-muscle axis and identifying gut microbial, metabolic and inflammatory signatures of sarcopenia in patients with cirrhosis.

2 | MATERIALS AND METHODS

2.1 | Patients selection and study procedures

All patients with cirrhosis consecutively admitted from 1 January to 31 June 2019 at the Hepatology Clinic of the Fondazione Policlinico

Universitario Agostino Gemelli IRCCS in Rome were assessed for eligibility. Inclusion criteria were as follows: age ≥ 18 years, absence of systemic or intestinal pathologies associated with gut microbiota alterations (eg celiac disease, inflammatory bowel diseases, diabetes mellitus etc) and complete abstinence from alcohol consumption for at least one year. Patients with previous or active tumours, chronic neurodegenerative or muscle diseases, use of probiotics, prebiotics or antibiotics during the previous 3 months, and those on vegetarian or vegan diet were also excluded. Actively exercising subjects were also excluded. A group of subjects without cirrhosis comparable for age and sex distribution and meeting the same eligibility criteria were enrolled as controls. The only comorbidities allowed for the control group were hypertension, prior stroke (more than 1 year before the enrolment) or non-obstructive peripheral vascular disease and mild chronic obstructive pulmonary disease. Among controls, a small group of sarcopenic subjects was also included to compare sarcopenic cirrhotic patients with sarcopenic controls.

The two participant groups received a nutritional evaluation, including a 7-day food frequency questionnaire, standard anthropometry and body composition analysis by whole-body dual X-ray absorptiometry (DXA). The diagnosis of sarcopenia was based on the presence of low muscle mass and strength, according to the criteria released by the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project,[7] as follows: (a) appendicular lean mass (ALM) to body mass index (BMI) ratio (ALM_{BMI}) < 0.789 and < 0.512 in men and women; or (b) crude ALM < 19.75 kg in men and < 15.02 kg in women when the ALM_{BMI} criterion was not met; and (c) handgrip strength < 26 kg for men or < 16 kg for women.

Faecal and blood samples were also collected for the analysis of the gut microbiota composition and metabolomics profile, as well as for the quantification of circulating cytokines/chemokines, markers of intestinal inflammation (calprotectin) and permeability (zonulin-1 [ZO1], lipopolysaccharide [LPS]). In particular, the panel of inflammatory cytokines measured in the present study was designed based on their involvement in pathways and processes relevant to cirrhosis and sarcopenia pathophysiology. A detailed description of laboratory techniques and procedures is provided in the Supplementary Methods.

The study was approved by the Ethics Committee of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS (protocol ID 741). All procedures were conducted in accordance with the principles laid down in the Declaration of Helsinki. A written informed consent was obtained from all participants prior to enrolment.

2.2 | Statistical analysis

Descriptive statistics were run on all data. Differences in demographic, anthropometric, clinical, functional characteristics, inflammatory and metabolic markers, and gut permeability/inflammation markers between cases and controls were assessed via Wilcoxon test and Fisher's exact test, for continuous and categorical variables respectively. Comparisons between cases and controls according to the presence of sarcopenia were performed by Kruskal-Wallis test with Dunn's post hoc analysis when appropriate and Fisher's exact test, for continuous and categorical variables respectively.

The gut microbiota alpha diversity in experimental groups was evaluated by the Chao1 index, which was calculated on raw data. Afterwards, data were processed to remove taxa not seen more than three times in at least 20% of samples and normalized by applying regularized logarithm transformation (rlog).[8] Principal coordinates analysis (PCoA) was performed on weighted UniFrac distance, using permutational multivariate analysis of variance on the distance matrix to unveil differences between cirrhotic patients and controls, as well as among subgroups of sarcopenic participants.

The analysis of gut microbial differential abundance at the phylum, family and genus level was carried out using a negative binomial distribution on raw counts normalized by 'size factors', taking into account sequencing depth between samples.[8] This method was chosen based on its good performance in experiments involving relatively small samples.[9] Differences in bacterial abundance were expressed as log2 fold change (log2FC); a log2FC higher or lower than ± 1.5 with a *P*-value < 0.05 adjusted for multiple comparisons (false discovery rate [FDR] control according to Benjamini-Hochberg method) was considered statistically significant.

Gut microbiota metabolomics analysis was conducted by Wilcoxon rank-sum test and then by multivariate partial least squares-discriminant analysis (PLSDA) after removing metabolites not seen in at least 25% of samples and normalizing raw data by quantile, log and pareto scaling. The model performance was estimated by the 10-fold cross validation method reporting the number of components (latent variables, LVs), accuracy, R2Y and Q2 values, and by permutation test (set at 2000 permutations) to confirm the statistically significant separation of the groups. Variable importance in projection (VIP) scores, summarizing the contribution of each metabolite to the model, were also plotted. Scores greater than 1 indicated the most relevant variables. Finally, metabolite set enrichment analysis (MSEA) and metabolic pathway analysis (MetPA) were used for the identification and the visualization of metabolic pathways enriched in cirrhotic patients compared with controls or in sarcopenic compared with nonsarcopenic cirrhotic patients, respectively. Metabolites were identified based on common chemical names, and annotations were verified using the human metabolome database (HMDB), Kyoto Encyclopedia of Genes and Genomes (KEGG), PubChem, chemical entities of biological interest (ChEBI), and METLIN databases. The library specific to *Homo sapiens* was chosen as reference. Global test algorithm was used for pathway analysis, while out-degree centrality was used for topological analysis. All p-values were adjusted for multiple testing using the FDR control according to the Benjamini-Hochberg method.

Finally, to obtain a multiomic picture of sarcopenia in cirrhosis, a correlation network based on Spearman's coefficients was built up, including gut bacteria, metabolites, inflammatory parameters and gut barrier markers found to be differentially represented in sarcopenic cirrhotic patients. The Girvan–Newman algorithm[10] was used to further detect cohesive subgroups of variables in the network, in order to unveil additional information on variables interconnection.

All of the analyses were performed using the R statistics program (version 3.6.2), Metaboanalyst (version 4.0) and Cytoscape (version 3.7.2).

3 | RESULTS

A total of 336 candidate participants, 187 with cirrhosis and 149 controls, were evaluated for inclusion. Of them, 236 were excluded for the following reasons: recent or active treatment with prebiotics, probiotics or antibiotics at the time of the evaluation (81 cases), history of active or previous cancer or chronic systemic or intestinal diseases potentially influencing the gut microbiota composition (60 cases), vegetarian or vegan diet or active alcohol consumption (32 cases), and refusal to be enrolled in the study (63 cases) (Figure S1). Finally, 50 cirrhotic patients and 50 controls were enrolled. The main characteristics of study participants are shown in Tables 1 and S1.

Cirrhotic patients had a median age of 69 years (61.3-76) and were mostly men (70%). The etiology of liver disease was related to hepatitis C virus (HCV) infection (40%), nonalcoholic fatty liver disease (NAFLD) (28%), hepatitis B virus infection (HBV) (16%) or alcohol abuse (16%). All patients affected by HCV-related cirrhosis had been previously treated with direct antiviral agents and had achieved a sustained viral response. All patients with HBV-related cirrhosis were treated with antiviral drugs (entecavir or tenofovir).

Sarcopenia was diagnosed in 19 (38%) cirrhotic patients. The main characteristics of cirrhotic patients and controls stratified according to the presence or absence of sarcopenia are also reported in Table 1. No differences were observed among groups for BMI or waist circumference. The weekly consumption of red meat, non-red meat, milk, fish, eggs, cured meats, cereals and bakery products, legumes, vegetables and fruits were similar between groups. Controls reported a higher intake of dairy products (cirrhotic patients vs controls P =.04; sarcopenic cirrhotic patients vs sarcopenic or nonsarcopenic controls P <.05).

3.1 | Gut microbiota composition in cirrhotic patients

The gut microbiota of cirrhotic patients showed a lower alpha diversity compared with controls (Chao 1 index 412.08 [316.12-535.63] vs 526.95 [418.67-642.94], P =.0009; Figure 1A) and clustered apart on the ordination plot (P =.001; Figure 1C). Mainly, the relative abundance of Proteobacteria (1.67, P <.0001), *Klebsiella* (3.732, P =.0006) and *Streptococcus* (1.73, P =.0008) was higher in cirrhotic patients than in controls, while *Methanobrevibacter* (−3.71, P <.0001) and *Akkermansia* (−1.76, P =.02) were decreased (Figure 2A and Table S2).

3.2 | Gut microbiota composition in sarcopenic vs non sarcopenic cirrhotic patients and controls

The gut microbiota alpha diversity of cirrhotic patients with sarcopenia (376.03 [314.52-439.49], Figure 1B) was lower relative to both their nonsarcopenic peers (463.35 [355.43-543.16] P =.04) and of sarcopenic controls (540.04 [450.64-616.38], P =.002). No difference in gut microbial alpha diversity was instead found within the control group (alpha diversity of nonsarcopenic controls 517.34 [406.71-643.71], P =.51). The ordination plot displayed a different gut microbial composition of the four groups (P =.03; Figure 1D).

Compared with nonsarcopenic cirrhotic patients, those with sarcopenia showed a lower relative abundance of *Prevotella* (−6.38, P <.0001), *Methanobrevibacter* (−2.5, P =.01) and *Akkermansia* (−1.96, P =.04), at the genus level, along with other differences (Figure 2B and Table S2). In contrast, cirrhotic patients with sarcopenia showed higher relative abundance of *Eggerthella* compared with their nonsarcopenic peers (3.24, P =.001) (Figure 2B and Table S2).

We also investigated whether the gut microbiota was different between sarcopenic cirrhotic patients and sarcopenic controls. Among all the differentially expressed components, *Klebsiella* (5.04, P =.0003) and *Streptococcus* (2.66, P =.005) were more abundant in sarcopenic cirrhotic patients. Instead, Veillonellaceae (−3.49, P =.002), Methanobacteriaceae (−3.74, P =.011), *Ruminococcus* (−1.84, P =.005) and *Dialister* (−4.42, P =.01) were lower in sarcopenic cirrhotic patients than in sarcopenic controls (Figure 2C and Table S2). The results of the comparisons among all subgroups are reported in Table S2.

3.3 | Intestinal permeability and systemic inflammation

We explored the integrity of the intestinal barrier using indirect markers of permeability/inflammation. As expected, serum levels of ZO1 (P =.0003) and LPS (P <.0001), and faecal concentration of calprotectin (P =.04) were higher in cirrhotic patients than in controls. However, no difference in any of these markers was observed between sarcopenic and nonsarcopenic cirrhotic patients (Table 2).

With regard to the serum inflammatory profile, sarcopenia in patients with cirrhosis was characterized by higher serum levels of interleukin (IL) 1 beta, IL2, IL6, granulocyte-macrophage-colony-stimulating factor (GM-CSF), C-X-C motif chemokine ligand (CXCL) 10, tumour necrosis factor (TNF) alpha and C-reactive protein (CRP).

Circulating fibroblast growth factor 21 (FGF21) levels were higher in cirrhotic patients with sarcopenia relative to their

TABLE 1 Demographic and clinical characteristic of cirrhotic patients and controls stratified according to the presence/absence of sarcopenia. Continuous variables are reported as median (IQR), categorical ones as frequencies (percentage). Statistically significant comparisons are highlighted in bold

	CIRRHOSIS Sarcopenia (19) group a	CIRRHOSIS no Sarcopenia (31) group b	CONTROLS Sarcopenia (14) group c	CONTROLS no Sarcopenia (36) group d	p-value*
Gender – male –female	12 (63.16) 7 (36.84)	23 (74.19) 8 (25.81)	8 (57.14) 6 (42.86)	21 (58.33) 15 (41.67)	.51
Age (years)	70 (63-74)	66 (58.5-76.5)	75.5 (72-77.25)	72.5 (58.25-75.25)	.18
Aetiology – HCV – NAFLD – HBV – alcohol	7 (36.84) 7 (36.84) 2 (10.53) 3 (15.79)	13 (41.94) 7 (22.58) 6 (19.35) 5 (16.13)	– – – –	– – – –	–
Haemoglobin (g/dL)	13.2 (12.2-14.1)	13.9 (12.3-15)	14.5 (13.7-16)	14.7 (13.9-16.2)	.26
WBC (mm^3)	5520 (4800-6400)	5900 (4700-6680)	7465 (5935-8922)	6210 (5240-7235)	.05
PLTs ($10^3/mm^3$)	136 (104-181)	148 (115-197)	210 (183.5-260.25)	220 (186-251.5)	**<.0001 ac, ad, bc, bd**
ALT (IU/L)	22 (20-31)	22 (20-26)	16.5 (14.25-23)	18.5 (16-24)	**.01 ac, ad, bc, bd**
Albumin (g/L)	40 (39-43)	43 (40-45.25)	40 (39-41)	41 (41-43)	**.02 bc**
Bilirubin (mg/dL)	1 (0.5-1.6)	0.9 (0.7-1.2)	0.7 (0.5-0.8)	0.75 (0.6-1)	**0.03 ac, bc**
INR	1.1 (1.05-1.27)	1.1 (1.04-1.21)	1.05 (0.96-1.05)	0.97 (0.85-1.04)	**.001 ad, bd**
MELD	8 (7-11)	8 (7-10)	–	–	–
Child-Pugh – A5 – A6 – B7	27 5 1	12 4 1	– – –	– – –	–
Waist circumference (cm)	107.5 (102-122)	102 (97-144)	114 (107-131)	105.25 (99-121)	.14
BMI (kg/m^2)	29 (25.48-30.91)	27.27 (24.36-29.12)	29.99 (29-31.79)	26.2 (24.39-28.68)	**.02 bc**
Red meat (weakly)	1 (1-2)	1 (0-1)	1 (1-2)	1 (1-2)	.25
Non-red meat (weakly)	1 (1-2)	2 (1-3)	2 (1-2)	2 (1-2.75)	.58
Milk (weakly)	2 (0-7)	7 (2-7)	7 (0.75-7)	7 (5.5-7)	.4
Fish (weakly)	1 (1-2)	1 (1-2)	2 (1-2)	1 (1-2)	.63
Eggs (weakly)	1 (1-1)	1 (1-2)	1.5 (1-2)	1 (1-2)	.52
Cured meats (weakly)	2 (1-2)	2 (1-2)	1 (0-2)	1 (1-1.75)	.79
Cereals and bakery products (weakly)	7 (7-7)	7 (7-7)	7 (7-7)	7 (7-7)	.41
Legumes (weakly)	1 (0-2)	2 (1-3)	1 (0-2)	1 (0.75-2)	.29
Vegetables (weakly)	7 (7-7)	7 (5-7)	7 (7-7)	7 (7-7)	.32
Fruits (weakly)	7 (7-7)	7 (7-7)	7 (7-7)	7 (7-7)	.27
Dairy products (weakly)	2 (1-3)	2 (1-3)	3 (1.75-4.25)	3.5 (2-4.75)	**.02 ac, ad**
Handgrip strength (kg)	23.37 (16.53-24.67)	31 (24.2-37.93)	18.35 (14.25-20.97)	29.88 (22.26-33.86)	**<.0001 ab, ac, bc, cd**
ALM (kg)	18.69 (15.19-20.11)	22.84 (17.63-25.42)	18.8 (15.71-21.5)	22.83 (16.98-23.93)	**.04 ab, ad, bc,**
ALM_{BMI}	0.69 (0.49-0.72)	0.82 (0.66-0.92)	0.51 (0.5-0.71)	0.81 (0.63-0.97)	**.0003 ab, ad, bc, cd**

Note: Nonalcoholic fatty liver disease (NAFLD); hepatitis C virus (HCV); hepatitis C virus (HBV); white blood cells (WBC); platelets (PLTs); alanine aminotransferase (ALT); international normalized ratio (INR); model for end-stage liver disease (MELD); body mass index (BMI); appendicular lean mass (ALM).

*: Overall p-value; statistically significant comparisons (P <.05) between the subgroup a, b, c and d are also specified.

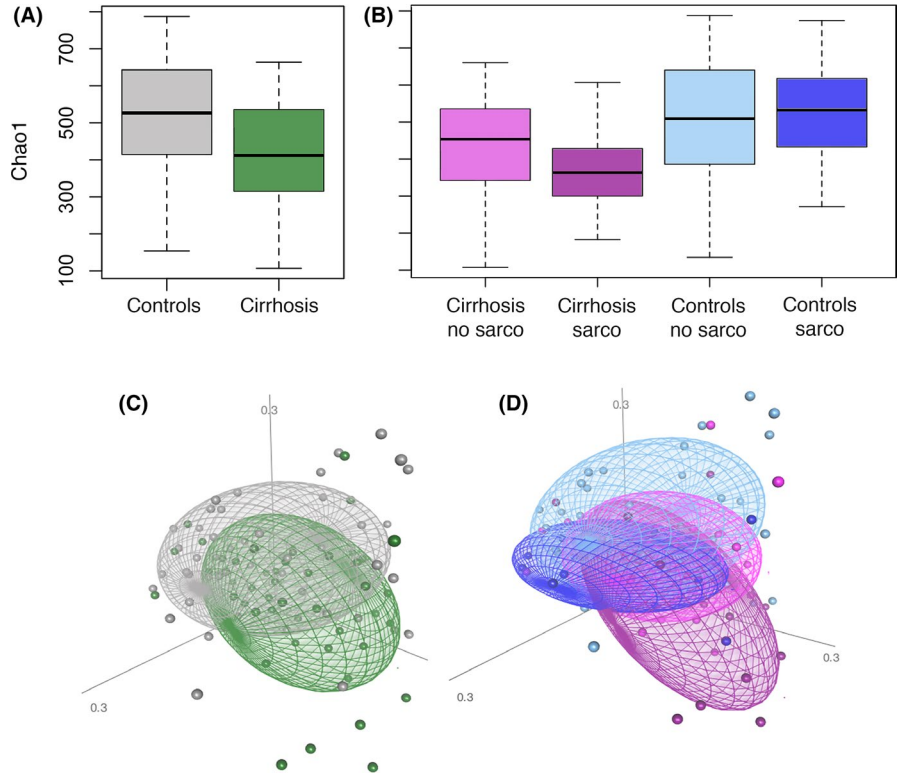

FIGURE 1 Box plots showing gut microbial alpha diversity (Chao1 index; panels A and B) and ordination plots displaying principal coordinates analysis (PCoA) on weighted Unifrac distance (panels C and D). In A and C, the whole population of cirrhotic patients is compared to controls, in B and D comparisons between patients and controls subgroups stratified according to sarcopenia are shown

nonsarcopenic counterparts (P <.0001), while myostatin, brain-derived neurotrophic factor (BDNF) and myeloperoxidase (MPO) serum levels were not significantly different.

3.4 | Characterization of the gut microbiota metabolomics of cirrhotic patients vs controls

To explore the relationship between the gut microbiota metabolomic profile and sarcopenia, we conducted untargeted faecal metabolomics analyses.

Univariate analysis (Table S3) showed a differential excretion of several metabolites between cirrhotic patients and controls; the two groups were also clearly separated at multivariate PLSDA (accuracy 0.91 R2Y 0.79 Q2 0.63, permutation test P <.0001 for the model including 2 components; Figure 3A). VIP scores > 1 were identified for the following metabolites: 2,3-pentanedione, arabinose, dimethylamine, orotate, succinate, N-methylnicotinate, xylose, valine, 4-hydroxyphenylacetate, methionine (all higher in the cirrhotic patients); butanal, 6-methyl-2-heptanone, 3-hexanone, hexadecane, 4-methyl-1H-indole (all higher in controls) (Figure 3B). These metabolites were functionally involved in several metabolic pathways, including aminoacyl-tRNA biosynthesis, valine, leucine and isoleucine biosynthesis and degradation, pantothenate and CoA biosynthesis, pentose and glucuronate interconversions, and cysteine and methionine metabolism (Figure 3C and Table S3b). Among all these pathways, cysteine and methionine metabolism showed the highest capacity in discriminating between cirrhotic patients and controls (P =.0002, impact 0.105) (Figure 3D, Table S3c).

3.5 | Characterization of the gut microbiota metabolomics of sarcopenic vs nonsarcopenic cirrhotic patients and controls

The gut microbiota metabolomic profile of cirrhotic patients with and without sarcopenia was clearly different at both univariate analysis (Table S4a) and multivariate PLSDA (accuracy 0.9 R2Y 0.96 Q2 0.62, permutation test P <.0001 for the model including three components; Figure 4A). Metabolites giving the most important contribution to the model (VIP score > 1) were the following: alpha-galactose, arabinose, glutamate, ethanol, alanine, xylose, trimethylamine (TMA) and dimethylamine (DMA) (all higher in sarcopenic cirrhotic patients); coapene, 2-pentanone, 4-methyl-1H-indole, p-cymene, isovalerate, butanoic acid ethyl ester, and 2-butanone (all higher in nonsarcopenic cirrhotic patients) (Figure 4B). The main metabolic pathways associated with cirrhosis and sarcopenia were: glycolysis/gluconeogenesis, pentose and glucuronate interconversions, alanine, aspartate and glutamate metabolism, seleno compound metabolism, and aminoacyl-tRNA biosynthesis (Figure 4C, Table S4b). The metabolome overview showed that valine, leucine and isoleucine biosynthesis (P =.008, impact 0.25), D-glutamine and D-glutamate metabolism (P =.008, impact 0.25), nitrogen metabolism (P =.008, impact 0.25), alanine, aspartate and glutamate metabolism (P =.001, impact 0.156) and arginine biosynthesis (P =.006, impact 0.125) were the most affected pathways in the group of sarcopenic cirrhotic patients (Figure 4D, Table S4c).

A metabolomic comparison between sarcopenic cirrhotic patients with sarcopenic controls was also performed. Results of PLSDA showed that the most important metabolites associated with sarcopenia in cirrhotic patients were arabinose, succinate, xylose,

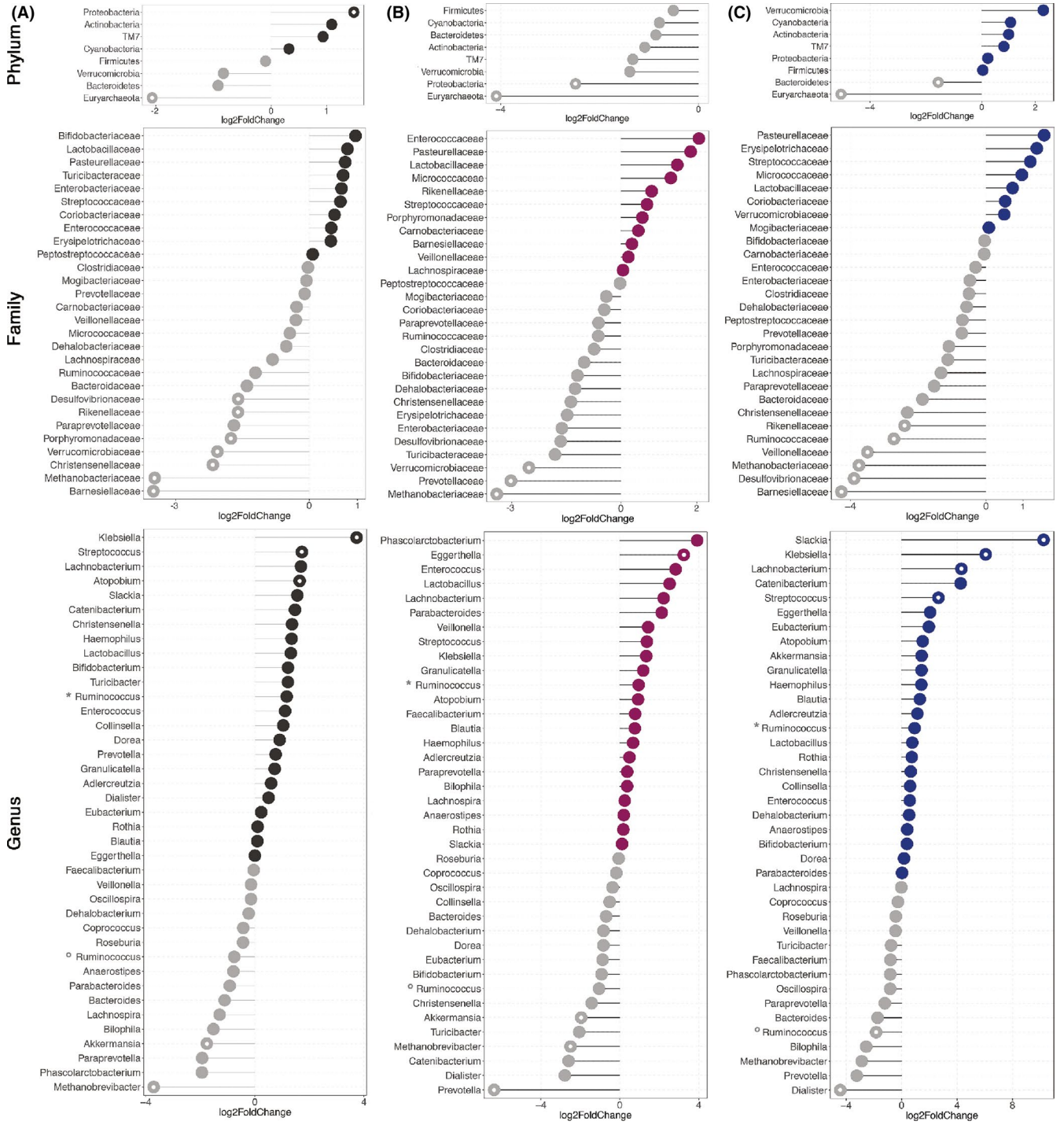

FIGURE 2 Differential abundance analysis of the gut microbiota composition at the phylum (upper panels), family (middle panels) and genus (lower panels) level between: cirrhotic patients vs controls (left, black colour); sarcopenic vs non sarcopenic cirrhotic patients (centre, purple colour); sarcopenic cirrhotic patients vs sarcopenic controls (right, blue colour). Differential bacterial abundance is expressed as log2 fold change (log2FC); positive or negative values indicate an increase or decrease proportional to the absolute value of log2FC. Comparisons with a log2FC higher or lower than ± 1.5 and a p-value < 0.05 adjusted for multiple comparisons with the Benjamini-Hochberg method (p-adj) were considered significant and are marked by a white spot inside the circle. *Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Ruminococcus. °Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus

alpha galactose, hypoxanthine, formate, dimethylamine, isoleucine and ethanol, while 2-pentanone, 2-nonanone, butanal, furfural, indole and p-cymene were the most represented in sarcopenic controls (accuracy 0.68 R2Y 0.76 Q2 0.18, permutation test P =.22 for the model including two components; Figure S2A and B). The metabolic pathways differentiating sarcopenic cirrhotic patients from sarcopenic controls were glycolysis/gluconeogenesis and butanoate metabolism (P =.03, impact 0.133) (Table S5 and Figure S2C and D).

TABLE 2 Markers of intestinal permeability, cytokines/chemokines, and myokines of cirrhotic patients and controls, also stratified according to the presence/absence of sarcopenia. Continuous variables are reported as median (IQR), categorical ones as frequencies (percentage). Statistically significant comparisons are highlighted in bold

	CIRRHOSIS (50)	CONTROLS (50)	p-value	CIRRHOSIS Sarcopenia (19) group a	CIRRHOSIS no Sarcopenia (31) group b	CONTROLS Sarcopenia (14) group c	CONTROLS no Sarcopenia (36) group d	p-value*
ZO1 (ng/mL)	130.05 (112.08-178.91)	108.33 (63.56-141.38)	**.0003**	128.09 (119.29-145.09)	148.35 (95.24-195.35)	142.62 (125.34-158.62)	88.39 (52.66-120.52)	**.0003 ad, bc, bd, cd**
LPS (EU/mL)	38.99 (23.73-207.36)	19.25 (14.50-29.21)	**<.0001**	49.10 (21.87-210.46)	37.47 (24.47-132.36)	17.77 (14.5-18.74)	24.87 (15.15-30.86)	**<.0001 ac, ad, bc**
Calprotectin (μg/g)	32.1 (18-66)	15.7 (9.2-35.36)	**.041**	35 (25.15-67.5)	31.1 (14.25-64.67)	31 (19-60.25)	11.8 (7.02-30.45)	**.040 ad, bd, cd**
IL1beta (pg/mL)	0.99 (0.68-1.59)	1.11 (0.93-1.66)	.335	1.59 (0.83-2.66)	0.91 (0.59-1.59)	1.11 (0.75-1.2)	1.15 (0.98-1.68)	**.026 ab, bd**
IL1ra (pg/mL)	94.72 (84.93-91.80)	110.33 (91.8-169.20)	.179	104.88 (90.35-232.43)	91.82 (73.18-122.34)	133.97 (86.91-169.82)	102.26 (91.81-156.96)	.248
IL2 (pg/mL)	6.04 (4.64-7.35)	6.535 (4.85-6.97)	.809	6.25 (5.63-8.64)	5.7 (4.58-6.25)	5.37 (3.53-6.14)	6.65 (5.68-7.07)	**.012 ab, ac, bd, cd**
IL4 (pg/mL)	3.11 (1.98-4.42)	2.37 (0.87-3.58)	.129	3.26 (1.81-4.92)	2.83 (2.10-4.16)	2.03 (1.00-3.62)	2.45 (0.86-3.56)	.399
IL6 (pg/mL)	1.23 (0.83-1.23)	2.37 (1.44-2.81)	**.002**	1.6 (1.23-2.99)	1.23 (0.71-1.78)	2.37 (1.87-2.99)	2.37 (1.23-2.81)	**.001 ab, bc, bd**
IL7 (pg/mL)	6.4 (3.5-9.32)	5.63 (0.86-7.46)	.180	6.4 (4.34-8.65)	6.4 (3.22-9.45)	4.37 (0.39-6.49)	6 (1.3-8.77)	.261
IL8 (pg/mL)	9.98 (7.1-23.84)	7.74 (6.09-13.28)	**.007**	13.95 (7.44-26.94)	9.88 (7.10-21.26)	7.70 (5.86-12.14)	7.74 (6.49-13.42)	**.042 ac, ad**
IL9 (pg/mL)	144.42 (51.42-170.25)	104.12 (60.38-116.44)	.151	158.91 (54.69-182.10)	143.66 (49.54-158.23)	110.06 (100.36-118.78)	97.75 (54.35-113.47)	.180
IL12 (pg/mL)	1.99 (0.00001-6.09)	2.81 (1.482-4.05)	.090	1.89 (0.00001-3.62)	2.32 (0.00001-1.89)	1.845 (0.84-2.81)	3 (1.52-4.72)	.170
IL17 (pg/mL)	15.69 (11.87-23.38)	18.13 (12.24-22.07)	.876	20.61 (13.87-24.84)	14.16 (10.87-21.95)	14.90 (12.63-20.32)	19.11 (12.09-22.58)	.505
CCL11 Eotaxin (pg/mL)	70.13 (57.17-104.97)	108.38 (66.28-268.28)	**.0009**	71.49 (55.69-111.74)	68.75 (57.37-100.7)	133.23 (78.96-304.16)	97.06 (65.35-238.9)	**.0054 ac, ad, bc, bd**
FGFb (pg/mL)	36.79 (32.24-41.66)	38.64 (30.99-52.48)	.269	40.45 (34.12-45.48)	35.51 (31.01-39.15)	42.11 (33.29-51.02)	38.64 (30.45-53.21)	.256
GCSF (pg/mL)	26.06 (13.37-38.72)	32.77 (12.29-47.45)	.127	47.11 (18.13-63.44)	25.5 (11.99-47.11)	24.87 (16.47-31.47)	26.06 (12.24-41.59)	.196
GMCSF (pg/mL)	1.86 (1.19-2.22)	1.41 (0.73-2.07)	.102	1.79 (1.04 −2.18)	1.02 (0.63-1.89)	1.56 (0.71-2.14)	1.86 (1.38-2.22)	**.035 ab, bd**
IFN gamma (pg/mL)	4.68 (4.22-7.9)	2.99 (2.5-4.68)	**<.0001**	5.28 (4.24-9.44)	4.36 (4.16-5.67)	2.5 (2.31-2.62)	3.6 (2.68-5.02)	**<.0001 ac, ad, bc, bd, cd**

(Continues)

TABLE 2 (Continued)

	CIRRHOSIS (50)	CONTROLS (50)	p-value	CIRRHOSIS Sarcopenia (19) group a	CIRRHOSIS no Sarcopenia (31) group b	CONTROLS Sarcopenia (14) group c	CONTROLS no Sarcopenia (36) group d	p-value*
CXCL10 IP10 (pg/mL)	536.71 (407.96-743.34)	560.52 (413.6-922.6)	.666	591.41 (503.06-1213.44)	476.9 (382.03-648.39)	801.77 (518.60-1129.14)	512.35 (347.8-752.93)	**.019 ab, bc, cd**
CCL2 MCP1 (pg/mL)	31.68 (19.07-54.7)	27.24 (16.92-35.88)	.156	36.48 (19.07-57.71)	29.48 (18.79-44.96)	30.175 (20.53-36.13)	26.58 (15.13-34.14)	.326
CCL3 MIP1 alpha (pg/mL)	1.96 (1.5-3.99)	2.57 (1.63-12.71)	.051	1.63 (1.5-4.02)	2.03 (1.42-3.87)	2.65 (2.32-12.71)	2.32 (1.5-11.99)	.207
CCL4 MIP1 beta (pg/mL)	109.53 (103.42-121.22)	122.12 (105.59-183.82)	**.028**	109.85 (103.74-140.06)	109.22 (103.45-118.17)	138.81 (92.44-183.07)	122.12 (111.04-181.77)	.134
PDGF beta (pg/mL)	1723.3 (233.37-3160.59)	2489.48 (393.63-3578.89)	.114	1534.38 (465.30-2877.94)	1873.69 (230.81-3264.23)	2467.44 (2176.24-3485.76)	2489.48 (274.87-3671.51)	.240
CCL5 RANTES (pg/mL)	8865.39 (4240.17-21028.38)	3080.34 (2122.91-13092.92)	**<.0001**	9512.25 (4725.35-24325.36)	7308.62 (4255.94-19953.91)	2393.12 (2102.68-12684.98)	3108.2 (2125.22-14178.57)	**<.0001 ac, ad, bc, bd**
TNF alpha (pg/mL)	41.31 (26.99-55.13)	26.09 (19.18-43.99)	**.013**	55.68 (33.3-104.92)	36.72 (25.495-44.92)	30.78 (19.91-43.64)	25.09 (18.93-42.93)	**.014 ab, ac, ad**
CRP (pg/mL)	1 450 315 (558297-3900573)	1 617 305 (1092344-3583303)	.446	2 877 837 (1 016 232.5-8975203)	1 318 001 (455 522.2-3187304)	5 019 855 (1 960 554.8-8682585)	1 385 835 (997 841.1-2173171)	**.001 ab, ad, bc, cd**
FGF21 (pg/mL)	120.56 (83.35-294.93)	269.05 (124.06-444.34)	**.008**	308.56 (183.69-339.27)	101.78 (74.25-125.98)	370.47 (203.35-500.37)	213.49 (70.08-407.54)	**<.0001 ab, bc, bd, cd**
Myostatin (ng/mL)	3.58 (2.69-5.31)	3.79 (2.93-5.09)	.931	4.27 (2.66-8.35)	3.37 (2.77-4.83)	5.17 (4.39-6.64)	3.23 (2.67-4.1)	**.007 bc, cd**
BDNF (pg/mL)	31 503.02 (20 668.55-38721.38)	36 799.08 (29 707.92-42161.96)	**.036**	31 472.71 (18 822.12-38091.15)	31 531.32 (22 437.85-38671.4)	38 208.05 (33 871.35-42488.76)	35 704.97 (28 222.61-40099.22)	.101
MPO (pg/mL)	443 210 (213 944.9-668827)	401 452.9 (227 099.5-843764.6)	.417	461 258.3 (219 585.3-720691.6)	437 789.2 (202 667.6-532876.3)	224 275.1 (190 133.6-253868.9)	557 396.6 (342 436.2-9731550)	**.006 ad, bd, cd**

Note: Zonulin-1 (ZO1); lipopolysaccharides (LPS); interleukin (IL); receptor agonist (ra); fibroblast growth factor (FGF); granulocyte colony-stimulating factor (G-CSF); granulocyte-macrophage-colony-stimulating factor (GM-CSF); interferon (IFN); C-C motif chemokine ligand (CCL); C-X-C motif chemokine ligand (CXCL); platelet-derived growth factor (PDGF); tumour necrosis factor (TNF); brain-derived neurotrophic factor (BDNF); myeloperoxidase (MPO).

*Overall p-value; statistically significant comparisons (P <.05) between the subgroup a, b, c and d are also specified.

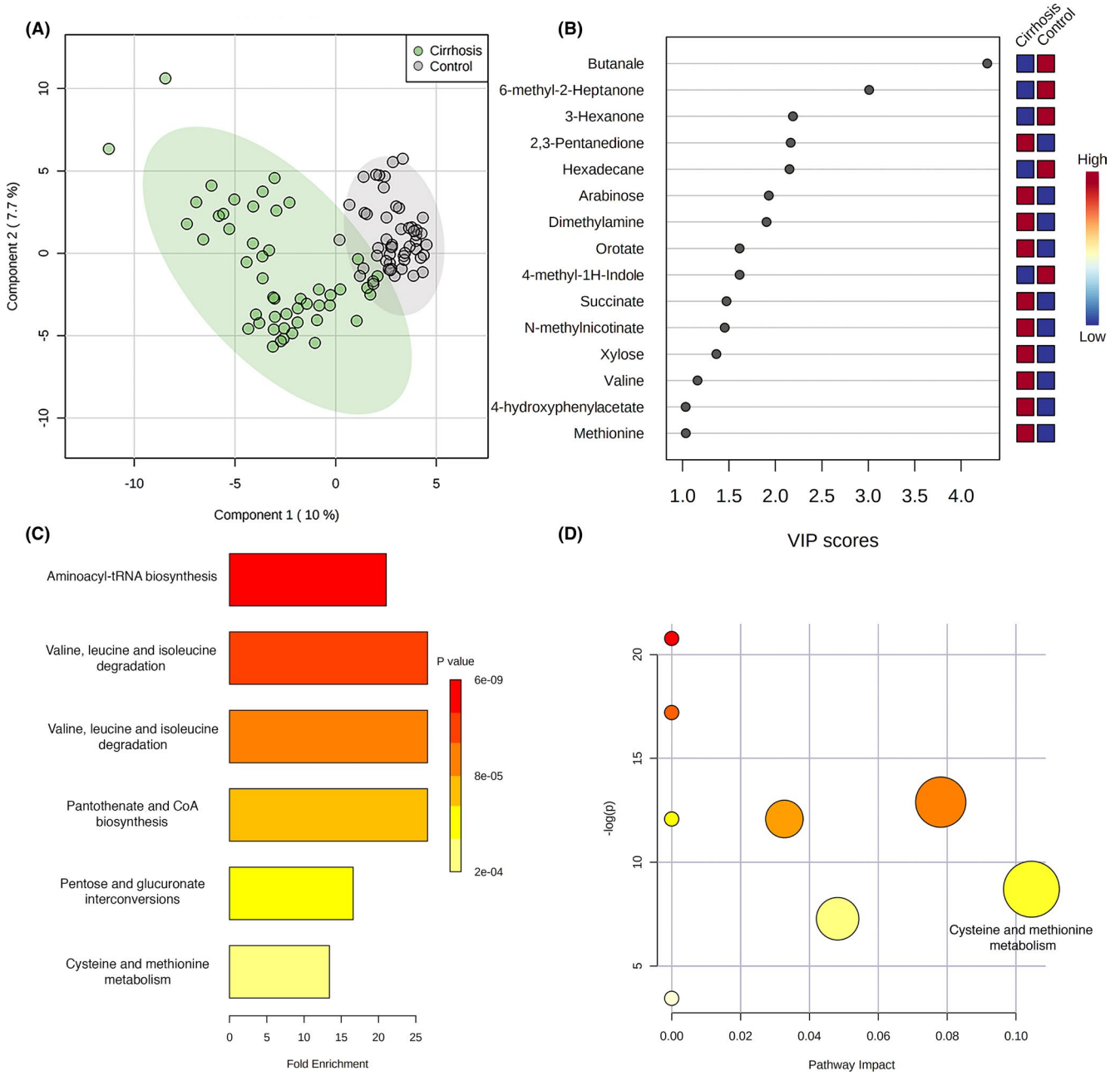

FIGURE 3 Gut microbiota metabolites analysis comparing cirrhotic patients and controls. A: partial least square discriminant analysis (PLSDA) of cirrhotic patients (green) and controls (grey); B: Variables important in projection (VIP) score plot of the most important metabolites contributing to the model. Increased metabolites are marked in red, decreased ones in blue (right column); C: Metabolic Set Enrichment Analysis (MSEA) showing the most altered metabolites in cirrhotic patients. The length of each bar is dependent on fold enrichment, the colour intensity (yellow to red) is proportional to statistical significance (p-value); D: Metabolic Pathway Analysis (MetPA) comparing cirrhotic patients vs controls. The − log p-value obtained from the pathway enrichment analysis is plotted on the y-axis, and the pathway impact value derived from the pathway topology analysis on the x-axis. Each metabolic pathway is represented by a circle; colour intensity (yellow to red) is proportional to the p-value, the radius to the pathway impact value

3.6 | Integrated approach to sarcopenia in cirrhotic patients

We finally combined data of differentially represented gut microbial components, intestinal barrier and inflammatory parameters, and gut microbiota metabolomics to explore their interconnection and networks correlated with sarcopenia in the group of cirrhotic patients.

Overall, 269 statistically significant correlations (Spearman's $r < -0.250$ or > 0.250 and adjusted P-value < 0.05) were observed (Table S6). The Girvan–Newman algorithm also allowed to identify three clusters of aggregation, the main nodes of which are displayed

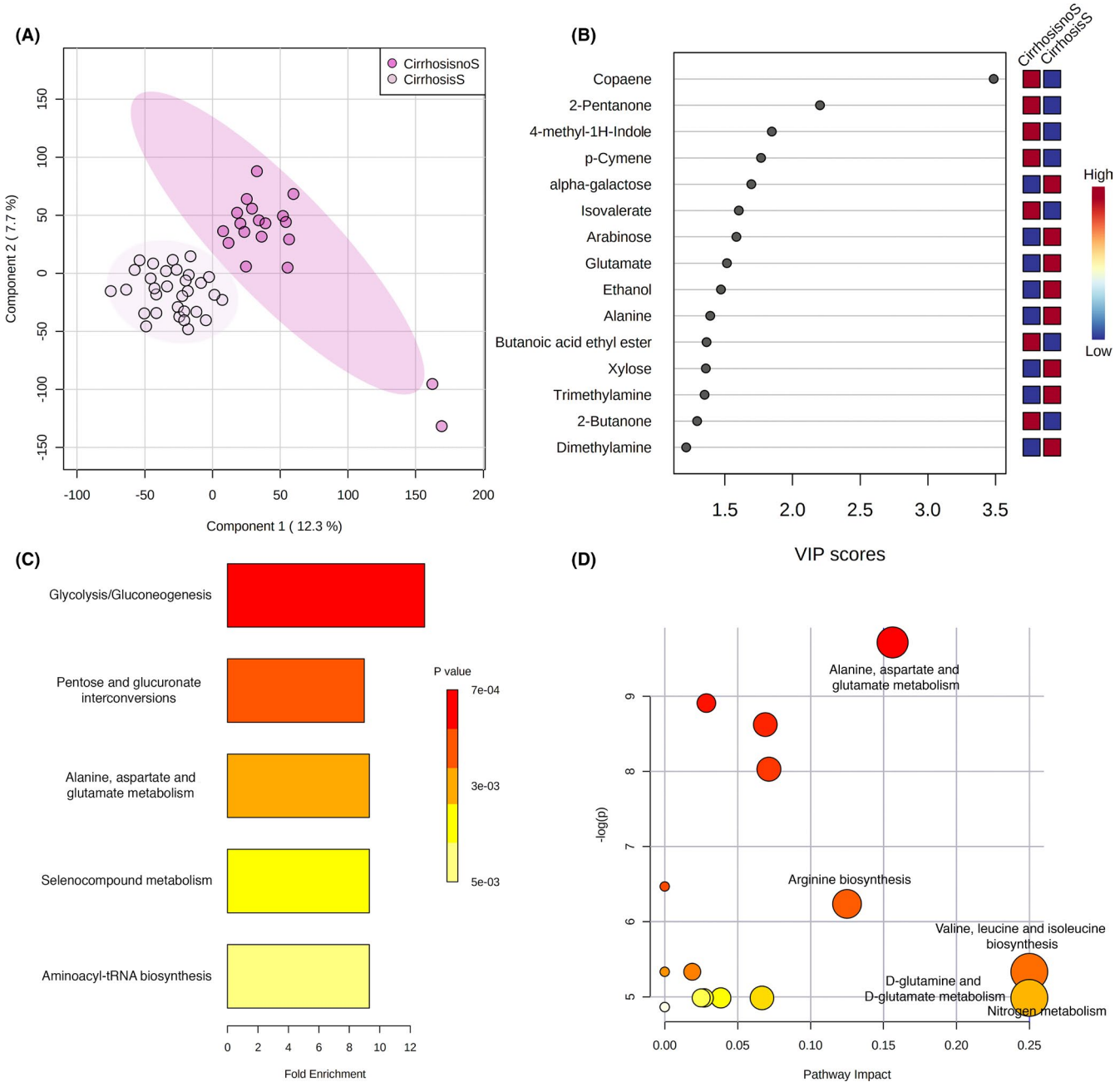

FIGURE 4 Gut microbiota metabolites analysis comparing sarcopenic and nonsarcopenic cirrhotic patients. A: partial least square discriminant analysis (PLSDA) of sarcopenic (pink) and nonsarcopenic (purple) cirrhotic patients; B: Variables important in projection (VIP) score plot of the most important metabolites contributing to the model. Increased metabolites are marked in red, decreased ones in blue (right column); C: Metabolic Set Enrichment Analysis (MSEA) showing the most altered metabolites in sarcopenic cirrhotic patients. The length of each bar is dependent on fold enrichment, the colour intensity (yellow to red) is proportional to statistical significance (p-value); D: Metabolic Pathway Analysis (MetPA) comparing sarcopenic vs nonsarcopenic cirrhotic patients. The − log p-value obtained from the pathway enrichment analysis is plotted on the y-axis, and the pathway impact value derived from the pathway topology analysis on the x-axis. Each metabolic pathway is represented by a circle; colour intensity (yellow to red) is proportional to the p-value, the radius to the pathway impact value

in Figure 5. Notably, factors associated with sarcopenia in cirrhotic patients were interconnected. The most relevant links identified between network nodes were: *Klebsiella*/ethanol/ALM_{BMI}/handgrip strength/FGF21/*Eggerthella*/*Prevotella*; *Methanobrevibacter*/valine/glutamate/methionine/TNF alpha/IL6/CCL4/CCL5/GMCSF/IL8; *Akkermansia*/*Streptococcus*/Proteobacteria/LPS.

4 | DISCUSSION

The gut microbiota is one of the main actors in the development of metabolic disorders. With this study, we have shown that gut dysbiosis is associated with sarcopenia in patients with cirrhosis.

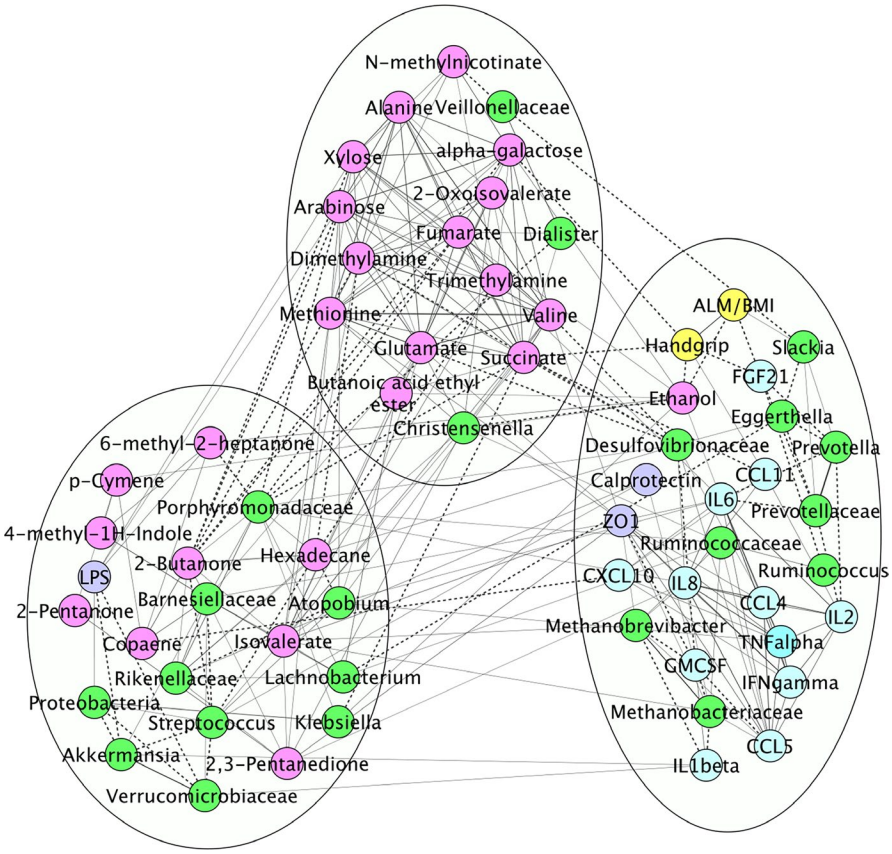

FIGURE 5 Correlation network including gut microbial components (green), faecal metabolites (pink), intestinal permeability markers (violet), cytokines/chemokines/myokines (light blue), handgrip strength and appendicular lean mass adjusted for body mass index (ALM/BMI) (yellow) differentially expressed in the group of sarcopenic cirrhotic patients. Only variables showing a Spearman's correlation coefficient < -0.25 or > 0.25 and adjusted p-value < 0.05 are shown. Each variable represents a node; positive correlations are highlighted by continuous lines, negative ones by dashed lines. Clusters of cohesive variables identified by the Girvan–Newman algorithm are highlighted by circles

Indeed, we observed a reduced alpha diversity and a different gut microbiota composition in sarcopenic cirrhotic patients compared with their nonsarcopenic counterparts as well as with sarcopenic controls. The detailed analysis of the gut microbial differential abundance showed a loss of Methanobacteriaceae, Prevotellaceae, Verrucomicrobiaceae, *Prevotella* and *Akkermansia* as well as an increase in *Eggerthella* in cirrhotic patients with sarcopenia compared with those without sarcopenia. Interestingly, people following a physically active lifestyle have a completely opposite gut microbiota composition, characterized by an increased relative abundance of *Methanobrevibacter*, *Prevotella* and *Akkermansia*, which is associated with favourable metabolic shifts in the gut microbial community.[11] In fact, *Methanobrevibacter smithii* promotes polysaccharide digestion by bacteria and fungi with the production of short chain fatty acids (SCFAs),[12] which are an important source of energy for the human organism and have been associated with greater lean body mass and better physical functioning.[13-15] The decrease in *Prevotella* is a renowned marker of frailty,[16-18] while its increase is associated with physical functioning and lean mass in older adults[19] and in young professional athletes.[20] Conversely, the genus *Eggerthella*, which was increased in sarcopenic cirrhotic patients, is overabundant in physical frailty, a condition that often overlaps with sarcopenia.[21,22] Conversely, only differences related to advanced liver disease, such as a lower abundance of Ruminococcaceae/*Ruminococcus*, Veillonellaceae and Rikenellaceae, and an increase in *Klebsiella* and *Streptococcus*, were observed by comparing sarcopenic cirrhotic patients with sarcopenic controls.[23,24]

Therefore, gut microbiota modifications associated with sarcopenia do not overlap with alterations distinctively caused by liver disease, thus possibly producing additional, unfavourable, effects. Interestingly, no difference was determined in the degree of alteration of intestinal barrier integrity or local inflammation between cirrhotic patients with and without sarcopenia. This observation suggests that the gut microbiota composition may be a more relevant factor for the development of inflammatory and metabolic changes associated with sarcopenia in cirrhosis than gut barrier impairment.

Sarcopenic cirrhotic patients also showed a pro-inflammatory cytokine profile which may further contribute to muscle atrophy. Indeed, sarcopenia is associated with persistent, low-grade inflammation,[25] which is shared also by cirrhosis, ageing, cancer and other diseases. In our sample, the inflammatory effects of dysbiosis associated with liver disease, and the lack of specific microbial components with anti-inflammatory properties such as *Akkermansia*,[26] may favour the over-production of several cytokines/chemokines expressed in cirrhotic patients, most of which have already been associated with sarcopenia.[27,28]

Furthermore, we found higher serum concentrations of FGF21 in sarcopenic cirrhotic patients. This finding might reflect a stress-induced increased secretion of FGF21 by the liver and/or the muscle in the setting of perturbed inter-organ crosstalk.[29] Notably, FGF21 has been shown to induce muscle atrophy via inhibition of protein synthesis and stimulation of autophagy.[29,30]

Another interesting finding of our study was the strict clustering of the gut microbiota and inflammatory features associated

with sarcopenia. Within this cluster, negative correlations were observed among ethanol, ALM_{BMI}, handgrip strength, FGF21, *Eggerthella* and *Prevotella* while a positive correlation was determined between *Klebsiella* and ethanol. An increased ethanol production by the gut microbiota has been already described in liver disease and is associated with its progression.[31,32] Furthermore, ethanol can affect muscle homeostasis by stimulating protein breakdown and autophagy.[33] Intestinal ethanol may result from either reduced clearance or an excessive production. Bacteria such as *Klebsiella*, which was enriched in our population of cirrhotic patients, act as ethanol producers.[34,35] Conversely, *Methanobrevibacter* and *Prevotella*, which were found to be deficient in sarcopenic cirrhotic patients, are involved in ethanol removal.[36,37] Thus, our findings support the involvement of ethanol in metabolic networks contributing to muscle atrophy in cirrhosis.

Furthermore, we observed an increased faecal concentration of TMA and its derivative DMA in sarcopenic cirrhotic patients. TMA is oxidized by the liver enzyme flavin-containing monooxygenase 3 (FMO3) into trimethylamine-N-oxide (TMAO), which is involved in atherosclerosis and cardiovascular disease.[38] The detrimental effects of TMAO are mediated by the induction of inflammation and oxidative stress in vascular endothelial and smooth muscle cells.[38] Whether TMAO can also contribute to the pathogenesis of sarcopenia in cirrhotic patients through the promotion of inflammation warrants investigation.

Pathways including metabolites involved in amino acid and protein metabolism, in particular glutamine, glutamate, alanine, aspartate, arginine, aminoacyl-tRNA biosynthesis and branched-chain amino acids (BCAAs) were also up-regulated in cirrhotic patients with sarcopenia. Liver impairment causes a shift from carbohydrates to amino acids as a source of energy, which contributes to muscle wasting.[39] In healthy people, half of the ammonia produced from this process is metabolized by the liver in the urea cycle, while the remaining ammonia is detoxified by the skeletal muscle.[40] Conversely, in patients with cirrhosis glutamine synthesis from glutamate in the muscle becomes the most important detoxification route.[40] This process involves BCAAs, such as valine, leucine and isoleucine, which are also relevant to muscle homeostasis.[41] While humans have no biosynthetic pathways for BCAAs, the gut microbiota can synthesize them. Hence, our results confirm the enrichment of the gut microbiota in bacteria involved in BCAA metabolism in cirrhotic patients, which was especially evident in those with sarcopenia.[42] Furthermore, our data indicate that, in patients with mild liver dysfunction, this 'gut microbial buffer' may support the gut-liver-muscle axis, as none of our patients presented high ammonia serum levels or clinical signs of hepatic encephalopathy.

Finally, we found that the gut microbiota of sarcopenic cirrhotic patients may contribute to the generation of intermediates of glycolysis/gluconeogenesis and pentose and glucuronate interconversions pathways, such as xylose and arabinose, as well as to the production of metabolites with antioxidant properties (ie arginine, a precursor of nitric oxide, from glutamate and selenocompounds from methionine). These metabolites may further support muscle homeostasis and counteract sarcopenia in cirrhotic patients.[43-47]

The present study has several strengths. First, we obtained a landscape of sarcopenia in patients with cirrhosis. We focused our analysis on patients with mild liver dysfunction, to capture the early metabolic and compositional changes of the gut microbiota that could contribute to the development of sarcopenia, avoiding the influence of other factors (eg hyperammonemia, malnutrition, inactivity) usually associated with a more advanced liver impairment. This information may be crucial in clinical practice, as it can help identify patients who may benefit from personalized treatments (eg gut microbiota modulation, anti-inflammatory treatments, nutritional support) to halt the progression of muscle wasting. The prevalence of cirrhosis in older adults has increased, and this is also confirmed by the median age of our patient population.[48] For this reason, we matched patients and controls by age and sex in order to exclude the effect of these factors on the results. Therefore, our findings open an interesting debate on whether changes in host metabolism or in the gut microbiota composition caused by diseases or clinical conditions other than aging share similar pathophysiological pathways leading to sarcopenia in different ways.

At the same time, our study has limitations. The original design did not include the analysis of plasma and urinary metabolites. Furthermore, the relatively strict eligibility criteria and the restriction of enrolment to Caucasian people, while allowing us to exclude confounding factors, could have limited the generalizability of our results. Finally, the metabolomic comparison between sarcopenic cirrhotic patients and sarcopenic controls was limited by the small sample size, thus additional analyses are needed to clarify the metabolic differences between these subjects.

In summary, a complex spectrum of alterations of the gut-liver-muscle axis may contribute to the development of sarcopenia in patients with cirrhosis. The loss of bacteria such as *Methanobrevibacter*, *Prevotella* and *Akkermansia* and the increase in *Eggerthella* and *Klebsiella*, installs a metabolic and pro-inflammatory network involving ethanol, TMA, myokines such as FGF21, cytokines and chemokines, which might ultimately contribute to muscle wasting. At the same time, the peculiar gut microbial composition in cirrhotic patients may act as a metabolic buffer, to limit muscle decay. Whether the dysfunction of the gut-liver-muscle axis is amenable for corrective interventions needs to be established to improve patients' outcomes.

5 | DATA AVAILABITITY STATEMENT

Data are available upon reasonable request and with permission of all the Authors.

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None.

CONFLICT OF INTEREST

None.

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REFERENCES

1. Dasarathy S, Merli M. Sarcopenia from mechanism to diagnosis and treatment in liver disease. *J Hepatol*. 2016;65:1232-1244.
2. Ponziani FR, Gasbarrini A. Sarcopenia in patients with advanced liver disease. *Curr Protein Pept Sci*. 2018;19:681-691.
3. Kim G, Kang SH, Kim MY, Baik SK. Prognostic value of sarcopenia in patients with liver cirrhosis: a systematic review and meta-analysis. *PLoS One*. 2017;12.
4. Bunchorntavakul C, Reddy KR. Review article: malnutrition/sarcopenia and frailty in patients with cirrhosis. *Aliment Pharmacol Ther*. 2020;51:64-77.
5. Carey EJ, Lai JC, Sonnenday C, et al. A North American expert opinion statement on sarcopenia in liver transplantation. *Hepatology*. 2019;70:1816-1829.
6. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol*. 2018;14:576-590.
7. Studenski SA, Peters KW, Alley DE, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *J Gerontol A Biol Sci Med Sci*. 2014;69:547-558.
8. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15:550.
9. Jonsson V, Österlund T, Nerman O, Kristiansson E. Statistical evaluation of methods for identification of differentially abundant genes in comparative metagenomics. *BMC Genom*. 2016;17:78.
10. Girvan M, Newman MEJ. Community structure in social and biological networks. *Proc Natl Acad Sci USA*. 2002;99:7821-7826.
11. Petersen LM, Bautista EJ, Nguyen H, et al. Community characteristics of the gut microbiomes of competitive cyclists. *Microbiome*. 2017;5:98.
12. Samuel BS, Gordon JI. A humanized gnotobiotic mouse model of host–archaeal–bacterial mutualism. *PNAS*. 2006;103:10011-10016.
13. Okamoto T, Morino K, Ugi S, et al. Microbiome potentiates endurance exercise through intestinal acetate production. *Am J Physiol Endocrinol Metab*. 2019;316:E956-E966.
14. Scheiman J, Luber JM, Chavkin TA, et al. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat Med*. 2019;25:1104-1109.
15. Lahiri S, Kim H, Garcia-Perez I, et al. The gut microbiota influences skeletal muscle mass and function in mice. *Sci Transl Med*. 2019;11.
16. van Tongeren SP, Slaets JPJ, Harmsen HJM, Welling GW. Fecal microbiota composition and frailty. *Appl Environ Microbiol*. 2005;71:6438-6442.
17. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. *Nature*. 2012;488:178-184.
18. Verdi S, Jackson MA, Beaumont M, et al. An investigation into physical frailty as a link between the gut microbiome and cognitive health. *Front Aging Neurosci*. 2018;10.
19. Fielding RA, Reeves AR, Jasuja R, Liu C, Barrett BB, Lustgarten MS. Muscle strength is increased in mice that are colonized with microbiota from high-functioning older adults. *Exp Gerontol*. 2019;127:110722.
20. Clarke SF, Murphy EF, O'Sullivan O, et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut*. 2014;63:1913-1920.
21. Jackson MA, Jeffery IB, Beaumont M, et al. Signatures of early frailty in the gut microbiota. *Genome Med*. 2016;8:8.
22. Cesari M, Landi F, Calvani R, et al. Rationale for a preliminary operational definition of physical frailty and sarcopenia in the SPRINTT trial. *Aging Clin Exp Res*. 2017;29:81-88.
23. Bajaj JS. Altered microbiota in cirrhosis and its relationship to the development of infection. *Clin Liver Dis (Hoboken)*. 2019;14:107-111.
24. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol*. 2014;60:940-947.
25. Casati M, Costa AS, Capitanio D, et al. The biological foundations of sarcopenia: established and promising markers. *Front Med (Lausanne)*. 2019;6:184.
26. Macchione IG, Lopetuso LR, Ianiro G, et al. Akkermansia muciniphila: key player in metabolic and gastrointestinal disorders. *Eur Rev Med Pharmacol Sci*. 2019;23:8075-8083.
27. Muñoz-Cánoves P, Scheele C, Pedersen BK, Serrano AL. Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *FEBS J*. 2013;280:4131-4148.
28. Marzetti E, Picca A, Marini F, et al. Inflammatory signatures in older persons with physical frailty and sarcopenia: the frailty "cytokinome" at its core. *Exp Gerontol*. 2019;122:129-138.
29. Luo Y, McKeehan WL. Stressed liver and muscle call on adipocytes with FGF21. *Front Endocrinol (Lausanne)*. 2013;4:194.
30. Oost LJ, Kustermann M, Armani A, Blaauw B, Romanello V. Fibroblast growth factor 21 controls mitophagy and muscle mass. *J Cachexia Sarcopenia Muscle*. 2019;10:630-642.
31. Yuan J, Chen C, Cui J, et al. Fatty liver disease caused by high-alcohol-producing Klebsiella pneumoniae. *Cell Metab*. 2019;30:675–688.e7.
32. Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology*. 2013;57:601-609.
33. Dasarathy J, McCullough AJ, Dasarathy S. Sarcopenia in alcoholic liver disease: clinical and molecular advances. *Alcohol Clin Exp Res*. 2017;41:1419-1431.
34. Wolfe AJ. Glycolysis for Microbiome Generation. *Microbiol Spectr*. 2015;3.
35. Clark DP. The fermentation pathways of Escherichia coli. *FEMS Microbiol Rev*. 1989;5:223-234.
36. Tsuruya A, Kuwahara A, Saito Y, et al. Major anaerobic bacteria responsible for the production of carcinogenic acetaldehyde from ethanol in the colon and rectum. *Alcohol*. 2016;51:395-401.
37. Samuel BS, Hansen EE, Manchester JK, et al. Genomic and metabolic adaptations of Methanobrevibacter smithii to the human gut. *Proc Natl Acad Sci USA*. 2007;104:10643-10648.
38. Liu Y, Dai M. Trimethylamine N-oxide generated by the gut microbiota is associated with vascular inflammation: new insights into atherosclerosis. *Mediators Inflamm*. 2020;2020:4634172.
39. Bojko M. Causes of Sarcopenia in liver cirrhosis. *Clin Liver Dis (Hoboken)*. 2019;14:167-170.
40. Olde Damink SWM, Jalan R, Dejong CHC. Interorgan ammonia trafficking in liver disease. *Metab Brain Dis*. 2009;24:169-181.
41. Khanna S, Gopalan S. Role of branched-chain amino acids in liver disease: the evidence for and against. *Curr Opin Clin Nutr Metab Care*. 2007;10:297-303.
42. Wei X, Jiang S, Chen Y, et al. Cirrhosis related functionality characteristic of the fecal microbiota as revealed by a metaproteomic approach. *BMC Gastroenterol*. 2016;16:121.
43. Portune KJ, Beaumont M, Davila A-M, Tomé D, Blachier F, Sanz Y. Gut microbiota role in dietary protein metabolism and

health-related outcomes: the two sides of the coin. *Trends Food Sci Technol*. 2016;57:213-232.
44. Nay K, Jollet M, Goustard B, et al. Gut bacteria are critical for optimal muscle function: a potential link with glucose homeostasis. *Am J Physiol Endocrinol Metab*. 2019;317:E158-E171.
45. Whanger PD. Selenium and its relationship to cancer: an update. *Br J Nutr*. 2004;91:11-28.
46. Calvani R, Miccheli A, Landi F, et al. Current nutritional recommendations and novel dietary strategies to manage sarcopenia. *J Frailty Aging*. 2013;2:38-53.
47. Samengo G, Avik A, Fedor B, et al. Age-related loss of nitric oxide synthase in skeletal muscle causes reductions in calpain S-nitrosylation that increase myofibril degradation and sarcopenia. *Aging Cell*. 2012;11:1036-1045.
48. GBD. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol*. 2020;5(3):245-266.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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