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Branched-chain amino acid supplementation does not improve measures of sarcopenia in cirrhosis: results of a randomised controlled trial

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Summary

Background: Sarcopenia is associated with adverse outcomes in cirrhosis. Branched-chain amino acids (BCAA) target several pathways that lead to muscle loss in this population.

Aims: We aimed to evaluate the impact of BCAA supplementation on sarcopenia measures in patients with cirrhosis.

Methods: We conducted a 12-month double-blinded, randomised, controlled trial of BCAA supplementation (30 g daily) compared to an equicaloric, equi-nitrogenous whey protein in volunteers with cirrhosis and reduced muscle strength. The primary endpoint was an increase in grip strength and upper limb lean mass measured on DEXA. Mean-adjusted differences (MAD, 95% CI) between groups at 6 and 12 months are reported as treatment effect using a linear mixed model for repeated measures.

Results: A total of 150 volunteers entered the trial (74 BCAA, 76 control), with a median age of 58 years [IQR 48; 63] and MELD of 14 [12; 17]. At 12 months, 57% in the BCAA arm and 61% in the control arm met the primary endpoint ($p = 0.80$). No significant between-group difference was found in grip strength (MAD -0.15 kg [−0.37; 0.06], $p = 0.29$) or upper limb lean mass (1.7 kg [−0.2; 3.6], $p = 0.22$) at 12 months. No significant differences in other body composition parameters, physical performance, frailty, rates of hospitalisation or mortality were found between the BCAA and the control group. Fatigue improved across the entire cohort, without significant between-group differences. 15% of volunteers reported side effects, with distaste higher in the BCAA arm ($p = 0.045$).

Conclusion: BCAA supplementation did not improve measures of muscle strength, mass or performance or physical frailty compared to a whey protein supplement in a randomised controlled setting. ACTRN12618000802202.

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1 | BACKGROUND

Sarcopenia, defined as reduced muscle mass and impaired muscle function, has established prognostic significance in cirrhosis and is an independent predictor of reduced survival primarily from sepsis-related mortality.[1,2] The pathogenesis of sarcopenia is multifaceted and many of the mechanisms that contribute to muscle dysfunction and loss are specific to the haemodynamic, metabolic and hormonal alterations that accompany cirrhosis.[3] Despite a wealth of literature examining its prognostic importance, few randomised controlled clinical trials have examined therapies for sarcopenia associated with chronic liver disease.

Branched-chain amino acids (BCAAs) include the three essential amino acids such as leucine, isoleucine and valine, which are characterised by the presence of an alaphatic side chain. They serve as a substrate for energy, protein and non-essential amino acid production and play a vital role in signalling functions promoting protein synthesis and muscle cell growth.[4] People with cirrhosis have an amino acid imbalance characterised by low circulating BCAAs and elevated aromatic amino acids. This results from reduced protein intake and enhanced BCAA catabolism through ammonia detoxification within skeletal muscle and use as an energy substrate. Low circulating BCAA levels are associated with hepatic encephalopathy, hypoalbuminaemia and sarcopenia.[5]

Randomised controlled data support the use of BCAA supplementation in cirrhosis to improve symptoms of recurrent hepatic encephalopathy[6] and to ameliorate hypoalbuminaemia.[7] Molecular and animal model studies report a trophic effect of BCAAs on skeletal muscle in cirrhosis.[8,9] However, results from single-arm prospective[7,10] and smaller randomised controlled trials[11–13] examining BCAAs as a therapy for sarcopenia in cirrhosis have been conflicting.

We hypothesised that BCAAs would improve sarcopenia in volunteers with cirrhosis who have muscle weakness. Therefore, we conducted a double-blind, randomised controlled trial of BCAAs compared to an equicaloric equi-nitrogenous whey protein to examine the effects on sarcopenia measures and outcomes in this population.

2 | METHODS

A double-blinded, randomised, controlled trial of BCAAs compared to an equicaloric, equi-nitrogenous protein in volunteers with cirrhosis was conducted at a single tertiary referral centre in Melbourne, Australia, between September 2018 and May 2022. The duration of the study was 12 months with study visits at baseline, 1, 3, 6, 9 and 12 months. The clinical trial was prospectively registered with the Australian and New Zealand Clinical Trials Registry (ANZCTR 12618000802202) and approved by the Austin Health Human Research Ethics Committee. All volunteers provided written informed consent prior to enrolment.

2.1 | Volunteers

Adult volunteers with a documented diagnosis of liver cirrhosis of any aetiology were recruited from outpatient liver clinics. Cirrhosis was determined by the treating clinician based on clinical, biochemical, radiological or histological measures. Inclusion criteria included volunteers aged 18–75 years with reduced HGS compared to age- and gender-based norms and those with ascites or a serum albumin level <35 g/L.[14] Non-dominant HGS was assessed as an average of three attempts using a Jamar® dynamometer. Radiological assessments of muscle mass were not included as a screening tool due to associated ionising radiation. Exclusion criteria included transplant-free survival <6 months as determined by the treating clinician, active alcohol intake, active untreated or advanced hepatocellular carcinoma and lactose intolerance.

2.2 | Intervention

Volunteers received 1 kg packages of powdered BCAAs or whey protein isolate sourced from Bioflex Nutrition Pty Ltd. The supplements were packed in plain packaging for blinding purposes. The BCAA supplement contained 448 kJ and 26.3 g protein and included 10.6 g leucine, 5.3 g of isoleucine and 5.3 g of valine (total BCAA content 21.2 g). The whey protein isolate contained 481 kJ and 26.6 g/day protein (total BCAA content 6 g). Participants were provided with a measured scoop of 30 g and instructed to take the supplement in 2–3 divided doses per day. Despite international recommendations, patients with cirrhosis rarely meet the recommended protein requirements of 1.2 g/kg/day for compensated cirrhosis and 1.2–1.5 g/kg/day in decompensated disease.[1,15] Therefore, an equicaloric, equi-nitrogenous control rather than a carbohydrate control was chosen to avoid confounding through differences in total daily protein intake.

Previous studies have used varied doses of BCAAs ranging from 10 to 30 g.[16] The prescription of a daily dose of 21.2 g of BCAAs was based on international guidelines for BCAA dosing in liver disease being 0.25 g/kg/day. In volunteers with a dry weight less than 50 kg, specialised dietitian input was sought, and the dose was reduced to a weight-based dose. Participants were asked to bring in remaining packages to scheduled study visits where they were weighed, and the remaining amount was documented to assess compliance. Participants were provided with additional packages at each study visit. Given the dose prescribed and long duration of follow-up, volunteers taking >50% of the supplement at 6 and 12 months were included in the per-protocol analysis.

2.3 | Randomisation

Eligible volunteers were randomised to BCAA or control in a 1:1 allocation. Groups were stratified for sex and severity of liver disease (MELD ≥15 vs. MELD <15). Austin Health clinical trials pharmacy

performed randomisation based on a permuted block design with a computer random number generator and block size of 4. Trial investigators and participants were blinded to the intervention, and the supplement was provided by pharmacy in identical plain packaging.

2.4 | Sarcopenia and body composition assessment

The primary composite endpoint was a 5% increase in non-dominant grip strength (HGS) and/or upper limb lean mass (ULLM) as measured by dual-energy x-ray absorptiometry (DEXA). A combined endpoint was used based on the consensus definition of sarcopenia that includes both muscle mass and strength parameters.[1] DEXA was used for muscle mass assessment as the primary endpoint rather than CT imaging due to its reproducibility and low-ionising radiation exposure for repeated measures. This was performed at 0-, 6- and 12-months postrandomisation. ULLM was used instead of appendicular lean mass (APLM) as DEXA is unable to differentiate lean tissue from fluid, and APLM may therefore be influenced by the presence of lower limb peripheral oedema.[17]

Secondary endpoints included changes in skeletal muscle area (SMA) and adipose tissue parameters measured by single slice CT taken at mid-third lumbar vertebrae (L3) at 0 and 12 months. Tissue segmentation was performed using Tomovision® software (Version 5.0, Toronto, Canada) based on Hounsfield units as previously described.[18]

2.5 | Physical performance and frailty

The short physical performance battery (SPPB), a combination of chair stands, balance and gait speed, was used to assess physical performance. Frailty was assessed using the Fried frailty index (FFI), encompassing five domains of weight loss, self-reported exhaustion, physical activity, strength assessment and gait speed.[19] Liver frailty index (LFI) was calculated based on HGS, chair stands and balance assessment and corrected for sex.[20]

2.6 | Dietary assessment

Dietary assessment based on a 24-h recall questionnaire was performed at each study visit to measure daily protein and dietary intake. All volunteers were provided with basic dietary advice for a high-protein high energy from a trained investigator aiming to achieve recommended daily intake of 1.2–1.5 g/kg of protein and 32kcal/kg.[3]

2.7 | Clinical and biochemical parameters

Clinical assessment including physical examination was performed at each study visit to document the presence of ascites and overt hepatic encephalopathy. Volunteers without overt hepatic encephalopathy were asked to perform a streamlined version of the Stroop test using EncephalApp on an iPad. Total *OffTime* plus *OnTime* was recorded, and the presence or absence of minimal hepatic encephalopathy was documented based on proposed cut-off values of >190 s.[21]

Bloods for haematology and biochemistry testing were collected at each study visit to enable calculation of model for end-stage liver disease (MELD) score and Child-Pugh Score. Ammonia levels were monitored at 3-monthly intervals. Given the association between sarcopenia and insulin resistance, percentage of glycosylated haemoglobin (HbA1c) was also measured.[22]

Hospitalisation duration and indication were recorded and cross-checked with medical records. Admissions for hepatic encephalopathy and sepsis, based on the International Sepsis Forum Consensus Conference on Definitions of Infection, were documented.[23]

2.8 | Immune function

The QuantiFERON Monitor levels, an interferon gamma release assay that measures both adaptive and innate immune function, were measured at 3-monthly intervals and analysed as previously described and as per the manufacturer guidelines.[24] This has been validated as a marker of infection risk in people with cirrhosis.[24]

2.9 | Quality of life

The chronic liver disease questionnaire (CLDQ) was conducted at baseline, 6 and 12 months. This is a validated questionnaire in cirrhosis that assesses domains including fatigue, activity, emotional function, abdominal and systemic symptoms and worry.[25]

2.10 | Sample size determination

To detect a difference at 12 months of 5% in ULLM and/or 5% in HGS between the treatment and control treatment group, using a one-sided *t*-test, with a *p*-value set at 0.05 and a power of 80%, the calculated sample size in each group was 67 subjects. With estimated 10% dropouts to 6 months, the sample size was set at 150 people.

2.11 | Statistical analysis

Baseline variables were presented as median plus interquartile range (IQR) for non-parametric data. Comparison of baseline demographics and numeric patient characteristics was based on Wilcoxon rank-sum test. Categorical variables were presented as number and frequency (%) and compared using chi-squared test or Fisher's exact test in cases of low frequency.

Treatment effect on primary and secondary endpoints followed the intention-to-treat principle (ITT). ITT analysis included all randomised subjects, and subjects were kept in their assigned groups. The treatment effect for main outcomes and most secondary outcomes was assessed using a repeated-measures linear mixed-effects model with restricted maximum likelihood estimates (REML). The four strata by MELD and sex used in the randomisation and baselines were present as covariable in the final analysis. Fixed effects in the model included the MELD strata, baseline levels, treatment group, time point (visit) and the interaction term of time point by treatment group. Random effects were included at the subject level. The treatment effect, represented by the interaction term, was quantified as the mean-adjusted between-group difference (MAD) with 95% confidence intervals (CIs) from baseline to 6 and 12 months, respectively. The significance level for the treatment effect was tested as a single *p*-value (*p* overall) over all time points using Kenward–Roger degrees of freedom. The mixed model supports a joint analysis of both between-subject variation and within-subject variation, as required to avoid the 'differences in nominal significance' (DINS) error of testing for changes from the first visit in separate groups. The mixed model is robust against missingness at random (MAR). Minor variations in multiple variables were inherent in and adjusted for at the subject in the model.

A sensitivity analysis was conducted per-protocol including only volunteers who were assessed to have adequate compliance over the study period and had outcome data available at all time points. Other sensitivity analyses were stratified by sex, compliance and recent hospitalisation for hepatic encephalopathy. Outcomes lacking repeated longitudinal measurements, such as the second follow-up visits (L3 CT body composition variables), were analysed using a linear model with generalised least squares, with group difference adjusted for MELD strata and baseline levels as the treatment effect. To permit intention-to-treat analysis in those outcomes, missing values had to be imputed. This was done in the presence of other body composition measures and the grouping factors by multiple chained equations and multivariate predictive mean matching in 100 data sets to obtain a pooled robust estimate of the treatment effect. Statistical significance was presented as the two-sided p-value over all time points. A $p < 0.05$ was considered statistically significant. The software package R, version 4.3.0 for Mac, was used to perform statistical analyses, with the added packages lme4 1-33, effects 4.2-2, mice 3.16.0 and mitml 0.4–5.[26–30]

3 | RESULTS

One hundred and fifty participants were recruited; 74 volunteers were randomised to receive BCAAs and 76 to the control protein supplement (Figure 1). One hundred and four (69.3%) volunteers were male, the median age was 58 years [IQR 48; 63], and most volunteers were Caucasian (Table 1). Table 1 summarises baseline demographics, which were well matched in both arms. Alcohol was the most common cause of cirrhosis and was responsible for liver disease in 40% of volunteers and a cofactor for liver disease in an additional 13%. All volunteers with chronic hepatitis C had achieved a sustained virological response to anti-viral therapy, with therapy completed at least 6 months prior to enrolment. No significant differences in cirrhosis aetiology were noted between the two groups.

Forty-three volunteers (28.7%) had Child-Pugh A, 51 (34%) had Child-Pugh B, and 56 (37.0%) had Child-Pugh C liver disease, and the median MELD score was 14 [12; 17] with no significant differences between the two arms (Table 1). Ascites requiring paracentesis within the past 3 months was present in 17.3% of volunteers (17.6% BCAA, 17.1% control) and 50.7% were prescribed diuretic therapy. No significant difference in the presence of encephalopathy

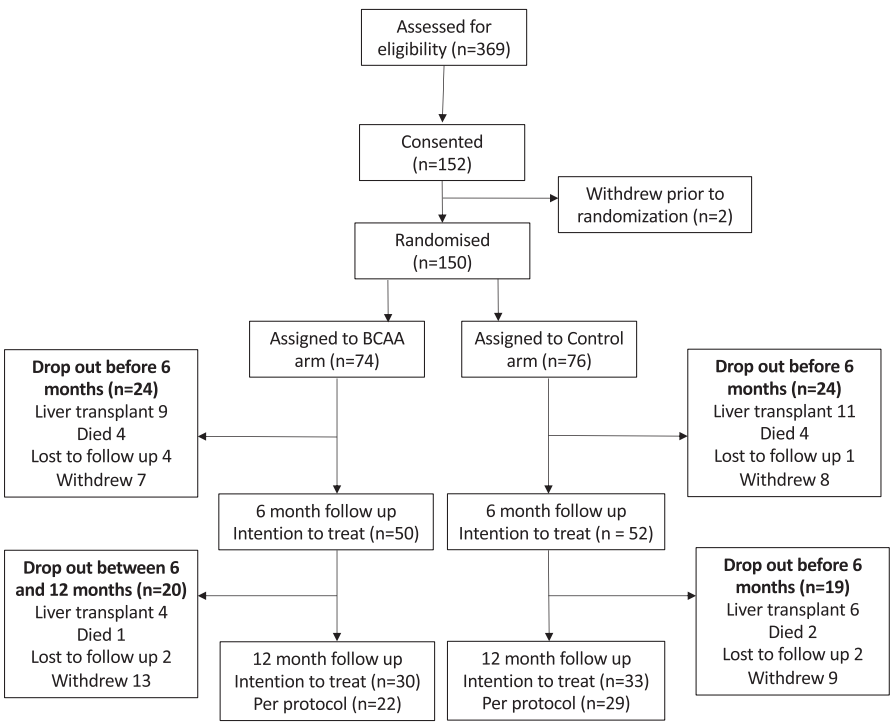

FIGURE 1 CONSORT diagram showing flow of patients randomised to branched-chain amino acids and control protein arm.

TABLE 1 Baseline variables.

	Control ($n=76$)	BCAA ($n=74$)	p-value
Age	58.5 [48; 63]	56 [45; 63]	0.37
Sex			
Male	52 (68.4%)	52 (70.2%)	
Female	24 (31.5%)	22 (29.7%)	
Ethnicity—Caucasian	64 (84.2)	66 (89.1)	0.43
Aetiology			
Alcohol	31 (40.8)	29 (39.1)	0.90
Viral hepatitis	18 (0.24)	21 (28.4)	
Non-alcoholic steatohepatitis	16 (21.1)	13 (17.6)	
Haemoglobin (g/L)	120 [111, 132]	117 [103, 131]	0.32
Platelets ($\times 10^9$/L)	90 [65, 139.5]	88 [61, 154]	0.97
Sodium (mmol/L)	138 [136, 140]	139 [136, 141]	0.20
Albumin (g/L)	30.5 [28.0, 33.5]	29.0 [26.0; 33.0]	0.10
Ammonia (μmol/L)	66 [52; 90]	58 [43; 77]	0.04
MELD	14 [11; 17]	14 [12; 17]	0.81
Child-Pugh Score	8 [6; 9]	8 [7; 9]	0.14
Child-Pugh Class			
A	27 (35.6)	16 (21.6)	0.11
B	21 (27.6)	30 (40.5)	
C	28 (36.8)	28 (37.8)	
Grip strength (kg)			
Male	30.2 [23.9, 35.9]	27.5 [23.3, 33.9]	0.67
Female	15.2 [12.1, 19.4]	18.8 [13.1, 22.9]	0.11
Skeletal muscle index (cm^2/height^2)			
Male	50.0 [42.4; 56.2]	50.2 [44.7, 56.1]	0.84
Female	42.3 [38.5; 46.6]	45.4 [38.4; 48.8]	0.51
APLM (kg)			
Male	22.7 [19.6; 24.8]	22.5 [20.3; 25.2]	0.81
Female	16.1 [14.5; 17.6]	18.3 [15.3; 23.1]	0.14
Short physical performance battery	9 [6; 11]	9 [7; 11]	0.56
Liver frailty index	4.26 [3.89; 4.75]	4.26 [3.77; 4.75]	0.63
Fried frailty index	2 [1; 3]	2.5 [2; 3]	0.34
Dietary intake			
Protein intake (g/kg/day)	0.9 [0.72; 1.01]	0.84 [0.72; 1.08]	0.79
Calorie intake (kcal/day)	1912 [1434.5; 2282]	1912 [1542; 2151]	0.97

at baseline ($p=0.24$) or recent hospitalisations for hepatic encephalopathy ($p=0.56$) was observed between groups. Baseline haematology, biochemical and muscle parameters were similar between the two arms except for blood ammonia levels, which were significantly higher at baseline in the control group.

3.1 | Study completion

One hundred and two volunteers (68.0%) completed 6-month follow-up (50 BCAA, 52 control), and 63 (42.0%) completed 12 months (30 BCAA, 33 control; Figure 1). By 12 months, 11 volunteers had died (5 BCAA, 6 control, $p=0.963$). The major reason for dropout was liver transplantation in 30 volunteers (20.0%). Other reasons for withdrawal included side effects or distaste in 12 (7 BCAA, 5 control), withdrawal of consent in 6 (3 per group), the development of other comorbidities (advanced malignancy, 3, acquired brain injury, 1) and inability to attend follow-up due to frequent hospitalisations from liver failure complications (2 BCAA, 3 control). Ten volunteers (6 BCAAs, 4 control) were unable to attend in-person follow-up appointments due to COVID-related travel restrictions in Victoria in place in 2020 and 2021. There was no significant difference in dropout rates per treatment arm.

3.2 | Primary endpoint

Baseline measures of HGS and ULLM mass were similar between treatment arms (Table 1). At 6 months, 29 (58.0%) and 27 (51.9%) of volunteers randomised to the BCAA and control arm, respectively, had reached the co-primary endpoint of a 5% increase in HGS and/or ULLM ($p=0.56$). By 12 months, 17 volunteers in the BCAA arm and 20 in the control arm achieved the primary endpoint ($p=0.80$). Examining each endpoint independently, changes in HGS and ULLM noted between groups were minor and statistically non-significant (MAD at 12 months of 1.7 kg [IQR −0.2; 3.6] and −0.15 kg [−0.37; 0.06], respectively; Table 2, Figure 2).

3.3 | Secondary endpoints: Body composition, frailty, and performance

At 6 and 12 months of enrolment, no meaningful or significant differences were noted in APLM, or lean mass between the BCAA and control groups (Table 2). No significant between-group differences were noted at 12 months on L3 CT body composition parameters including SMA, subcutaneous fat area and visceral fat area, frailty (liver frailty index and Fried frailty index) and the short physical performance battery.

3.4 | Haematology, biochemistry and immune function

No significant differences were found between the control and BCAA group on biochemistry or haematology. In particular, no difference in serum albumin level was noted in the BCAA arm compared to control (MAD at 6 months −0.34 g/L [−1.56, 0.88] and 12 months −0.24 g/L [−1.69; 1.21], $p=0.86$). No significant between-group differences were noted in ammonia, HbA1c or QuantiFERON Monitor levels at 6 or 12 months.

3.5 | Quality of life

There was no between-group difference noted in the total or the activity component of the CLDQ (Figure 3). For the fatigue component of the CLDQ questionnaire, there was no treatment effect found between arms ($p=0.66$), although there was an overall improvement across the entire cohort.

3.6 | Compliance

At 6 months, 74.0% and 88.5% of volunteers were considered compliant in the BCAA and control group, respectively ($p=0.11$). This was similar at 12 months (73.3% vs. 87.9%, $p=0.25$). When examining total product consumed, the control group had consumed a higher number of bags of trial product than the BCAA group at 6 months (control: 4.2 bags [3.8; 4.3] vs. BCAA: 3.8 [3.3; 4.2], $p=0.03$). This was not observed at 12 months (control: 3.6 bags [3.3; 3.9] vs. BCAA 3.2 [2.8; 3.6], $p=0.18$). Despite this, daily dietary protein intake increased similarly across both groups, with no significant between-group difference ($p=0.99$; Figure 4). At baseline, 16.0% and 14.5% in the BCAA and the control group, respectively, met the recommended daily protein requirement of 1.2 g/kg. This had increased to 50% and 54.9% at 6 months and 65.5 and 61.3% (BCAA vs. control) by 12 months. There was no association between volunteers who achieved the primary endpoint and those whose daily protein intake met recommended amounts of ≥1.2 g/kg at 6 or 12 months ($p=0.46$ and 0.83, respectively). There was an overall improvement in daily caloric intake across the entire cohort over the 12 months, with no significant between-group differences ($p=0.20$; Figure 4).

3.7 | Clinical outcomes

There were no significant differences in rates of all-cause hospitalisations or hospitalisations for hepatic encephalopathy ($p=0.22$) or sepsis ($p=0.38$) between the BCAA and control protein arms. No significant differences were noted in Stroop test or trail-making test results. There was no difference in mortality rates in BCAA compared to control-treated subjects (6.7% vs. 7.9%, $p=0.80$).

3.8 | Sensitivity analysis

A per-protocol analysis was performed based on study completers who were deemed compliant over the study period. This was further stratified by sex. No significant between-group differences in sarcopenia, frailty or performance parameters were found (Table 3). No other differences in Stroop test, haematology or biochemistry were noted. There were no differences between groups in sarcopenia measures in volunteers with and without a history of recent hepatic encephalopathy. Compliance with study product did not influence HGS ($p=0.37$) or ULLM ($p=0.47$) results.

An exploratory analysis of volunteers who completed to 6 months of follow-up (completers) compared to those who dropped out prior (non-completers) was performed. Non-completers had more severe liver disease, lower muscle mass and worse performance on Stroop testing (Table 4). No significant differences, however, were found between frailty or strength measures between volunteers who did and did not complete 6-month follow-up. Overall, grip strength of volunteers who reached 6- and 12-month follow-up remained stable (mean difference 0.52 kg [95% CI −10.36, 12.1] at 6 months and 0.55 kg [−8.01, 10.17] at 12 months). ULLM was similar with a mean difference of −0.07 kg (−1.7, 1.07) at 6 months and 0.01 g (−1.44, 1.15 kg) at 12 months. Importantly, completers and non-completers were balanced between the treatment groups. An analysis was also conducted between volunteers who achieved the primary endpoint at 12 months (responders) compared to non-responders. Overall, responders had

TABLE 2 Mean-adjusted difference in the primary and secondary endpoints of body composition, frailty and performance measures by intention-to-treat analysis.

	Control ($n = 76$)	BCAA group ($n = 74$)	Mean-adjusted difference[a] (MAD) [95% CI]	Overall p-value
Primary endpoints				
Grip strength(kg)				
0 months	24.0 [18.1; 32.4]	25.4 [20.3; 32.2]		0.22
6 months	22.4 [18.6; 30.9]	27.4 [21.2; 31.2]	0.8 [−0.9; 2.4]	
12 months	25.1 [18.5; 31.7]	27.2 [21.2; 35.7]	1.7 [−0.2; 3.6]	
Upper limb lean mass (kg)				
0 months	5.00 [3.99; 5.98]	5.17 [4.41; 6.33]		0.29
6 months	5.08 [3.81; 6.26]	5.64 [4.53; 6.48]	−0.11 [−0.29; 0.07]	
12 months	5.12 [4.01; 6.49]	5.46 [4.52; 6.25]	−0.15 [−0.37; 0.06]	
Secondary endpoints				
Appendicular lean mass (kg)				
0 months	20.43 [16.89; 24.13]	21.65 [18.60; 24.03]		0.87
6 months	20.82 [16.18; 24.90]	22.25 [18.26; 26.33]	−0.13 [−0.68; 0.42]	
12 months	20.65 [16.89; 23.94]	22.11 [18.79; 24.93]	−0.14 [−0.79; 0.51]	
Lean mass (kg)				
0 months	53.3 [44.1; 60.2]	54.8 [47.2; 59.5]		0.49
6 months	53.1 [43.9; 59.45]	54.9 [49.9; 63.5]	−0.7 [−2.1; 0.7]	
12 months	51.0 [43.5; 56.7]	55.3 [46.3; 59.4]	0.3 [−1.4; 2.0]	
Fat mass (kg)				
0 months	25.5 [19.2; 31.5]	25.2 [19.1; 33.7]		0.91
6 months	26.7 [19.8; 33.3]	26.8 [21.1; 35.4]	−0.2 | [−1.7, 1.2]	
12 months	25.3 [19.3; 32.0]	22.9 [20.0; 31.0]	−0.3 | [−2.1, 1.4]	
Skeletal muscle area (cm^2)				
0 months	139.2 [114.4; 160.7]	139.2 [121.2; 161.4]		0.21
12 months	151.4 [112.2; 166.0]	150.7 [137.2; 164.1]	−4.7 [−12.3; 2.8]	
Subcutaneous fat area (cm^2)				
0 months	180.5 [96.8; 263.8]	180.9 [106.7; 272.1]		0.17
12 months	167.3 [106.2; 251.3]	161.9 [134.5; 246.2]	24.8 [−11.6; 61.2]	
Fried frailty index				
0 months	2 [1; 3]	2.5 [2; 3]		0.15
6 months	2 [1; 3]	2 [1; 3]	−0.3 [−0.7; 0.1]	
12 months	2 [1; 3]	2 [1; 2]	−0.4 [0.8; 0.1]	
Liver frailty index				
0 months	4.26 [3.89; 4.75]	4.26 [3.77; 4.75]		0.43
6 months	4.29 [3.83; 4.78]	3.98 [3.66; 4.74]	−0.08 [−0.26; 0.10]	
12 months	4.14 [3.73; 4.41]	4.03 [3.69; 4.46]	−0.13 [−0.35; 0.08]	
Short physical performance battery				
0 months	9 [6; 11]	9 [7; 11]		0.76
6 months	10 [6; 11]	11 [7; 12]	0.3 [−0.5; 1.0]	
12 months	10 [9; 12]	10 [6; 12]	0.0 [−0.9; 0.8]	

Note: Crude data presented as the median [interquartile range]. Outcomes represent the mean-adjusted difference (MAD), surrounded by its 95% confidence interval, in branched-chain amino acid supplement arm, compared to the control protein arm.

[a]Refer to Methods for estimation of MADs and overall p-value.

a lower baseline MELD score (14±4 vs. 15±4, $p=0.004$) and Child-Pugh Score (7±2 vs. 8±2, $p=0.041$) than non-responders. No other differences in age, biochemistry, a history of hepatic encephalopathy, the presence of ascites or BCAA use were found.

3.9 | Safety and tolerance

Twenty-two volunteers (14.7%) reported side effects of protein supplements (11 per arm). Gastrointestinal side effects included nausea ($n=7$), vomiting (2) and bloating (1) did not differ between groups. Vomiting was self-resolving in one patient and persistent in one resulting in trial withdrawal. Distaste was more common in the BCAA than control group (13.5% vs. 3.9%, $p=0.045$). Other side effects included loss of appetite in six volunteers (4 BCAA, 2 control) and weight gain in one patient. Eleven volunteers (7.3%) died during the study period (6 BCAA, 5 control, $p=0.96$). Causes of death included progressive decompensated liver failure (5), acute variceal haemorrhage (2), cardiac arrest (2), ischaemic colitis (1) and intracranial haemorrhage (1).

4 | DISCUSSION

In this 12-month double-blinded randomised controlled trial of volunteers with cirrhosis and muscle weakness, we report that BCAA

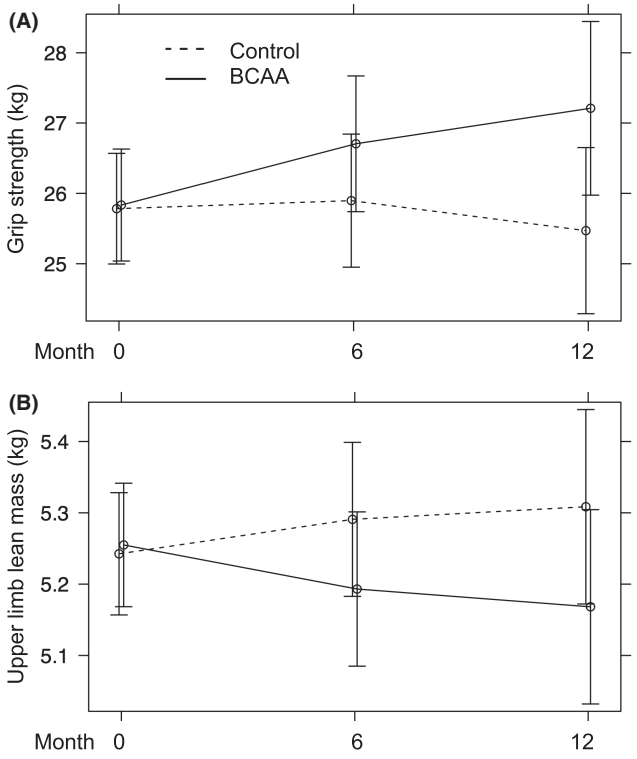

FIGURE 2 Mean-adjusted difference in (A) upper limb lean mass and (B) grip strength between the branched-chain amino acid and control protein arms.

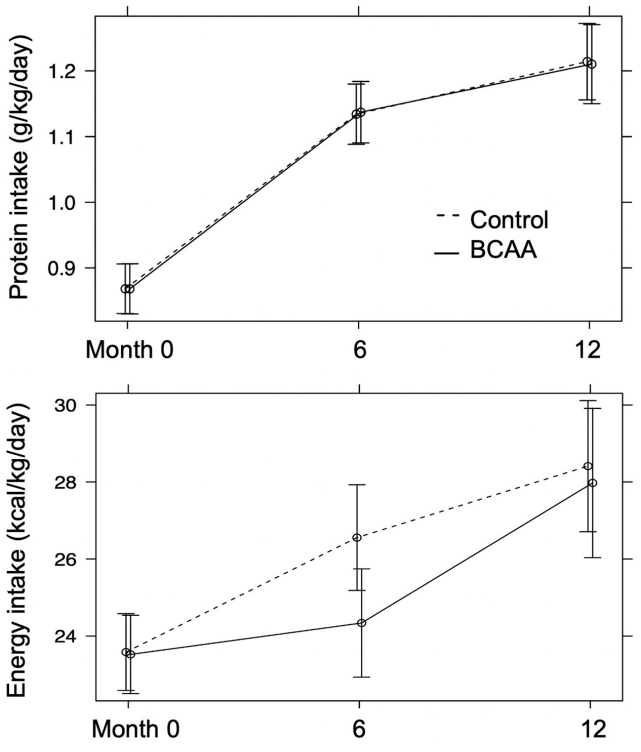

FIGURE 4 Mean-adjusted difference in daily protein and energy intake.

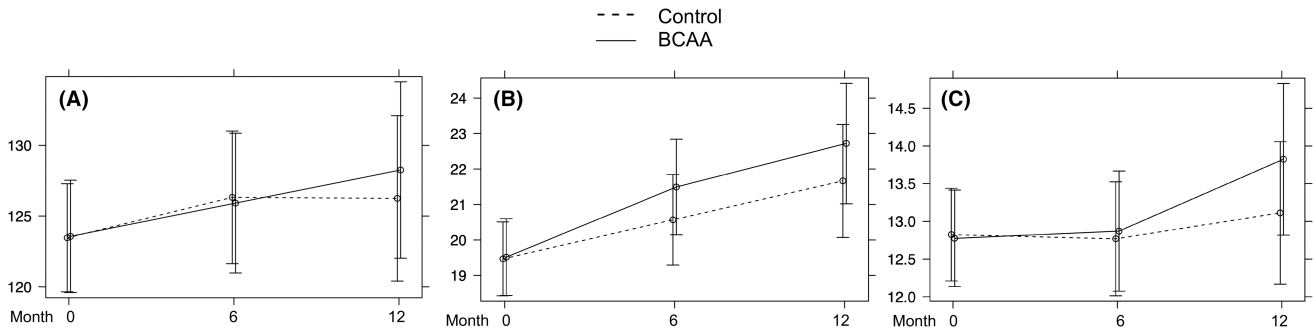

FIGURE 3 Mean-adjusted difference in quality of life. Measured using the chronic liver disease questionnaire. (A) represents total score, (B) fatigue component, (C) activity component.

TABLE 3 Per-protocol analysis based on completers who demonstrated compliance.

	MAD at 6 months ($n=22$)	MAD at 12 months ($n=29$)	p-value
Grip strength (kg)	1.4 [−1.4; 4.2]	2.80 [−0.0, 5.6]	0.16
Men	1.6 [−1.7; 4.9]	3.1 [−0.3; 6.4]	0.22
Women	0.9 [−4.5; 6.3]	2.0 [−3.4; 7.5]	0.78
Upper limb lean mass (kg)	−0.01 [−0.35; 0.33]	−0.08 [−0.42; 0.26]	0.89
Men	0.07 [−0.38; 0.52]	−0.01 [−0.46; 0.44]	0.93
Women	−0.28 [−0.64; 0.09]	−0.31 [−0.68; 0.05]	0.24
Appendicular lean mass (kg)	0.08 [−0.73; 0.88]	0.23 [−0.58; 1.03]	0.86
Total Lean mass (kg)	0.46 [−1.48; 2.41]	0.88 [−1.07; 2.82]	0.68
Fat mass (kg)	0.94 [−1.76; 3.65]	0.03 [−2.68; 2.73]	0.75
Fried frailty index	−0.7 [−1.3; −0.1]	−0.6 [−1.2; 0.0]	0.05
Liver frailty index	−0.16 [−0.43; 0.12]	−0.31 [−0.59; −0.03]	0.10
Short physical performance battery	0.5 [−0.7; 1.7]	0.4 [−0.7; 1.6]	0.48
Albumin (g/L)	−0.67 [−2.78; 1.43]	0.22 [−1.89; 2.32]	0.70
Ammonia level (μmol/L)	8 [−13; 29]	8 [−14; 29]	0.71
QuantiFERON monitor (IU/mL)	−6.1 [−135.0; 122.8]	30.3 [−98.6; 159.2]	0.84

TABLE 4 Comparison of baseline parameters between completers and non-completers to 6-month follow-up.

	Completers ($n=102$)	Non-completers ($n=48$)	p-value
Grip strength (kg)	24.3 [19.2; 31.3]	25.2 [18.6; 32.4]	0.77
Upper limb lean mass (kg)	5.29 [4.31; 6.54]	4.79 [3.96; 5.82]	0.045
MELD score	13.5 [11, 17]	16 [12.5; 19]	0.006
Child-Pugh Score	7.5 [6, 9]	8 [7, 10]	0.011
Sodium	136 [133; 139]	139 [137; 141]	<0.001
Skeletal muscle index (cm^2/height^2)	48.8 [42.2; 55.3]	45.4 [39.5; 50.7]	0.029
Liver frailty index	4.28 [3.77; 5.13]	4.22 [3.86; 4.68]	0.53
Stroop test (on time + off time)	166.15 [144.45; 210.10]	191.31 [170.29, 227.93]	0.007
BCAA treatment	50 (49%)	24 (50%)	0.91

Abbreviations: BCAA, branched-chain amino acids; MELD, model for end-stage liver disease.

supplementation did not significantly improve measures of sarcopenia compared to a control protein. The dropout rate was higher than expected, highlighting the difficulties faced in designing interventional trials for sarcopenia in this population. Although compliance was imperfect, findings from the ITT and per-protocol analysis were similar. This study finds no evidence that BCAA supplementation is superior to standard whey-based protein supplementation in people with cirrhosis and muscle weakness.

To our knowledge, this trial is the largest and longest duration to directly examine the effects of BCAAs on muscle and frailty parameters in a cirrhotic cohort. Results from three recent published randomised trials in this area have been conflicting. An unblinded study of 106 cirrhotic patients compared daily BCAAs to lactalbumin for 24 weeks.[13] Patients receiving BCAAs demonstrated an increase in total abdominal muscle area measured on magnetic resonance imaging, grip strength and gait speed compared to the lactalbumin-treated group. Similarly, Hernandez-Conde et al. conducted a double-blinded pilot study of BCAAs compared to a carbohydrate placebo for 12 weeks in addition to exercise.[11] Of 32 patients reported in the per-protocol analysis, the BCAA group demonstrated a significant improvement in SMI compared to placebo (2.3 kg vs. 0.04 kg, $p=0.022$). Mohta et al. randomised 60 cirrhotic patients to receive BCAA or a carbohydrate placebo in addition to a home-based exercise programme.[12] Similar to our results, after 6 months of therapy, intention-to-treat and per-protocol analysis revealed no significant differences in skeletal muscle index, grip strength or gait speed between groups.

Our study protocol and population differed to these trials. We included volunteers with all severity of liver disease and the

duration of this trial was longer. Our higher dropout rate likely relates to the sicker cohort of people included and longer duration of follow-up. We selected an equicaloric, equi-nitrogenous protein as the control arm to ensure blinding and to minimise confounding introduced by differences in overall dietary protein intake. Unlike the other studies, we assessed differences in estimated daily protein intake across the study period. Despite imperfect compliance, volunteers in both groups significantly increased total daily protein intake and most volunteers who reached at least 6-month follow-up achieved the minimum recommended daily protein intake of 1.2 g/kg. Our study therefore specifically examined the use of BCAA supplementation as a protein source as opposed to other trials where differences in total protein intake may have influenced results.[11,12]

The strongest evidence to support the administration of BCAAs in cirrhosis stems from trials examining their use in hepatic encephalopathy. A Cochrane review of 16 randomised trials with 827 participants concluded that BCAA administration had a beneficial effect on encephalopathy measures (RR 0.74, 95% CI 0.61–0.88).[6] Sarcopenia and hepatic encephalopathy are closely linked. Skeletal muscle is a biologically active tissue that acts as a major site for extra-hepatic ammonia detoxification via the tricarboxylic acid (TCA) cycle, and low muscle mass is an established risk factor for the development of encephalopathy. However, the process of ammonia detoxification induces cataplerosis within skeletal muscle, which drives muscle loss. BCAA replenishes key intermediates within the TCA cycle enabling improved muscle ammonia clearance.[31] This is the proposed mechanism by which BCAAs may improve encephalopathy. On sensitivity analysis, we found that men treated with BCAAs demonstrated an improvement in trail-making test time compared to the control group. This was not found in the group as a whole and may reflect the fact that this study was not designed and powered to examined encephalopathy as a primary endpoint.

BCAAs have several proposed anabolic effects on skeletal muscle. Leucine activates the intra-cellular mammalian target of rapamycin complex 1 pathway that initiates myofibrillar protein synthesis and downregulates the ubiquitin proteasome autophagy–lysosome systems responsible for protein degradation.[8,32] The combined effect of these stimulates muscle hypertrophy. As mentioned, BCAAs enhance extra-hepatic ammonia clearance in states of hyperammonaemia. In addition to skeletal muscle cataplerosis, hyperammonaemia induces myostatin, a myokine that negatively regulates muscle, and promotes mitochondrial dysfunction and autophagy with skeletal muscle cells.[33] These toxic effects of ammonia on skeletal muscle are reversed by the administration of leucine-enriched BCAAs.[31] Despite these proposed mechanisms, we observed no substantial improvement in muscle mass or strength overall or compared to a control protein in our trial.

The pathogenesis of sarcopenia in cirrhosis is complex, with multiple contributing factors beyond malnutrition alone. Hormonal alterations, hyperammonaemia, altered energy utilisation and portal hypertension, which drives inflammation and malabsorption all contribute to muscle loss and dysfunction. Cirrhosis is associated with anabolic resistance driven by increased myostatin expression, where muscle protein synthesis demonstrates a blunted response to anabolic stimuli such as protein and exercise. These factors may contribute to the lack of improvement in sarcopenia measures observed overall and between arms in our study. Lai et al. reported that people waitlisted for liver transplantation had a mean decline in grip strength of 0.38 kg every 3 months.[34] Decline in muscle strength was associated with adverse outcomes and higher waitlist mortality. Reassuringly, in our study, completers demonstrated stable muscle mass and strength parameters, while they remained on the trial. This suggests that while optimisation of protein intake does not improve sarcopenia it may attenuate the natural progression of muscle dysfunction and muscle loss.

The impact of BCAAs on mortality and quality of life remain unclear. An early, landmark, multicentre trial of 174 people with cirrhosis found BCAA administration was associated with a reduced combined event rate, hospitalisations and improved quality of life compared to lactalbumin or maltodextrin.[35] Similar to our trial, the Cochrane review found no improvement in mortality or quality of life in patients with encephalopathy treated with BCAAs.[6] These divergent results have led to differing recommendations by societal and consensus guidelines. European guidelines recommend the prescription of 0.25 g/kg/day of BCAAs in patients with advanced cirrhosis to improve event-free survival and quality of life.[36] In contrast, the American Association for the Study of Liver Disease (AASLD) does not advise the use of BCAAs beyond achieving daily recommended protein intake targets.[37] Our results failed to show an improvement in quality of life or mortality in BCAA compared to control protein-treated volunteers, although it was not appropriately powered to assess the latter.

The major limitation to this study was the higher-than-expected dropout rate, although non-completers remained balanced across the treatment groups. Unfortunately, this reflects the inherent difficulty in studying people with end-stage liver disease, who often progress to death or the requirement for liver transplant. There is a paucity of data surrounding the expected benefit of BCAA supplementation on muscle parameters, and several assumptions were made in the power calculation. There is a possibility that this, combined with the high dropout rate to 12 months, may have led to a type 2 error and the possibility of missing a small true treatment effect. We included a heterogeneous group of people with cirrhosis who had varying severity and aetiologies of liver disease and potentially different underlying contributors to sarcopenia. Regardless of intervention group, people who met the primary endpoint at 12 months had milder liver disease than those who did not, suggesting that more advanced liver disease may be associated with increased anabolic resistance in response to protein supplementation. Importantly, severity of liver disease was well balanced across treatment groups.

24-hour recall was used to estimate dietary intake due to frequent use in the cirrhotic literature, the short time required and applicability to larger studies.[38] This however is prone to recall bias and underreporting particularly in the setting of hepatic encephalopathy. The inherent differences in exercise capacity across a

diverse population made it difficult to design a specific home-based exercise programme that was suitable for all participants. Therefore, only general advice was given regarding exercise, with potential between-group differences in physical activity during the study a potential source of confounding.

This study has several major strengths. It is the largest randomised controlled trial to examine the effects of BCAAs on sarcopenia in cirrhosis. We included comprehensive sarcopenia endpoints including muscle mass, function, performance and frailty, all of which are associated with increased mortality in advanced liver disease. Muscle mass and strength are non-linear and may have sex differences in prognostic importance.[39] Despite this, sex-stratified results are underreported in studies examining sarcopenia in cirrhosis. In our trial, we randomised by and adjusted for sex and performed a sensitivity analysis based on sex which did not influence results. Blinding and the use of a control protein rather than a carbohydrate placebo as used in other trials[11,12] are in keeping with established recommendations to ensure participants achieve the minimum recommended daily protein intake of 1.2 g/kg/day. This allowed us to assess the impact of BCAA more specifically as an intervention while demonstrably removing the potential confounding by differences in total protein intake.

In conclusion, in this double-blind randomised controlled trial, BCAAs did not substantially improve measures of sarcopenia, frailty or quality of life compared to a control protein matched for total grams of protein. Ultimately, these findings in addition to the lower palatability of the BCAA supplement support AASLD guidelines that the most important target for a patient with cirrhosis is to meet their recommended daily protein intake. Subgroups of patients, particularly those with more severe encephalopathy, may specifically benefit from BCAA supplementation, but there is, as yet, no strong evidence to support their use for sarcopenia therapy alone.

AUTHOR CONTRIBUTIONS

Penelope Hey: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; writing – original draft; writing – review and editing. **Rudolf Hoermann:** Formal analysis; writing – review and editing. **Marie Sinclair:** Conceptualization; funding acquisition; methodology; supervision; writing – review and editing. **Brooke Chapman:** Conceptualization; investigation; writing – review and editing. **Adam Testro:** Funding acquisition; supervision; writing – review and editing. **Ross Apostolov:** Project administration; writing – review and editing. **Peter Angus:** Methodology; writing – review and editing. **Paul Gow:** Conceptualization; methodology; supervision; writing – review and editing.

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