SHORT-TERM EFFECT OF WHEY PROTEIN SUPPLEMENTATION ON THE NUTRITIONAL STATUS AND QUALITY OF LIFE OF PATIENTS WAITING FOR LIVER TRANSPLANTATION

Abstract:

Background & aims: Chronic liver disease is associated with malnutrition that negatively impacts a patient's health-related quality of life (HRQoL). We evaluated the short-term effect of whey protein supplementation on the HRQoL and nutritional and functional status of patients waiting for liver transplantation (LT). **Methods:** This was a double-blind randomized clinical trial with patients waiting for LT who were randomized into two groups: WP (whey protein supplementation) and the control (casein supplementation). Both groups received 40g (20g in the morning and 20 g in the evening) for 15 days. Nutritional and functional status were evaluated. Energy balance (EB) was calculated as the difference between energy intake (24-hour recall) and total energy expenditure. The chronic liver disease questionnaire (CLDQ) was used to assess HRQoL. All measurements were performed before and after the intervention. **Results:** Fifty-six patients were evaluated. Malnutrition was present in 56.9% of patients, and it was directly associated with a poor HRQoL ($p<0.05$). No improvement on the nutritional and functional status was observed, in either group after protein supplementation. HRQoL improved after WP and casein supplementation, with no differences between groups ($p>0.05$). Patients who met protein requirements and had a positive EB demonstrated a higher HRQoL score (4.9) ($p<0.05$), without between-group differences. **Conclusion:** Malnutrition substantially reduces HRQoL. Short-term WP or casein supplementation improved the HRQoL.

Keyword: Health-related quality of life, whey protein, nutritional status, chronic liver disease

Introduction

Liver transplantation (LT) is the only treatment for patients with end-stage liver disease and acute liver failure [1], offering them the chance to increase their life expectancy, and often leading to a better quality of life [2]. However, long waiting list time until the operation, particularly in developing countries, negatively impacts patients' quality of life[3] and their overall clinical status. These patients commonly present several complications associated with the evolution of liver disease, such as anorexia, early satiety due ascites and portal hypertension, asthenia, esophageal varices, and hepatic encephalopathy[4], which interfere with their nutritional and functional status, and thus with their health-related quality of life (HRQoL)[5].

HRQoL refers to the results of subjective and dynamic assessments of a patient's self-perception of state of health. It integrates physical, mental, and social contexts related to health[6], in addition to changes in functional capacity, daily activities, and emotional relationships in the patient's life that can cause a decrease in HRQoL[7].

Previous studies have shown that the quality of life of patients with cirrhosis is significantly lower than that of healthy individuals[8-11]. These findings have been associated with the severity of liver disease and vary according to changes in the patients' clinical and nutritional status. They also worsen the functional capacity, leading to impairment of daily living activities and HRQoL[5, 12].

Considering the relationship between malnutrition and HRQoL, nutritional interventions should be mandatory as an integral therapeutic approach for liver transplant patients[8, 9]. Nutritional therapy, focused on the ingestion of an adequate

amount of protein, has been associated with improved liver function and increased nutritional and functional status, and thus a consequent improvement in the quality of life of chronic liver disease and cirrhosis patients[13, 14]. Besides increasing the amount of protein, it is essential to consider the quality of protein to promote adequate muscle synthesis[10]. In this regard, supplementation of high-quality protein such as milk proteins (casein or whey protein), might be an interesting strategy, as they provide essential amino acids directly involved with muscle protein synthesis, consequently improving the overall nutritional status [15].

The positive effects of milk proteins on nutritional status are well established[16, 17]. These proteins are composed of different functional and physiological bioactive peptides, related to their structure and amino acid composition, which have recently been found to be associated with health improvements [18]. However, whey protein, compared with casein, has a higher digestibility and an increased amount of branched-chain amino acids, especially leucine [19], which favor a fast serum amino acid increase, which is considered an indispensable mechanism in protein synthesis[20]. This protein synthesis affects the antioxidant and immune systems as well as neurological functions[21], suggesting that whey protein may be more effective than casein in treating chronic liver disease, and consequently, improving the quality of life.

This study aimed to evaluate the short-term effect of whey protein supplementation on the HRQoL and nutritional and functional status of patients awaiting liver transplantation.

Materials and methods

This was a double-blind randomized clinical trial, carried out with patients awaiting liver transplantation at a Public Hospital. Patients of both sexes, aged 18 years,

and who were active on the waiting list for LT were invited to participate. Pregnant or breastfeeding women, as well as individuals with advanced kidney disease and those awaiting retransplantation were excluded. All patients gave written informed consent. The study was conducted according to the Declaration of Helsinki and was approved by the local University Ethics committee (CAAE-27430714.8.0000.5149) and registered at Clinical Trials (NCT02901119).

Patients were randomly assigned in a 1:1 ratio by computer-generated random numbers into two groups: intervention (WP) and control (C) based on the source of protein supplementation, that is, whey protein or casein, respectively. Participants were assessed by the researchers at two different times: a) First evaluation – full clinical history, nutritional and functional evaluations, 24-hour recall (R24h), resting energy expenditure (REE) assessment, and quality of life questionnaire. Further, in this first assessment, patients received the supplements and orientation on how to prepare and consume them. b) Second evaluation – 15 days after the first evaluation – the patients underwent the same assessments as in the initial moment, and 24 h recall was collected. Their supplement intake was monitored through weekly telephone calls, and they were instructed to bring the empty packages of supplements on the return visit. Each patient received 30 sachets containing 20 g of whey protein or casein, in each unit, to be taken twice a day, one in the morning and one in the evening, diluted in 150 mL of water or juice. The patients were instructed to maintain consistent eating and living routines during the study. Whey protein and casein sachets appeared similar. The patients and researchers were blinded to the intervention.

The nutritional status of the patients was assessed by Subjective Global Assessment (SGA)[22]. Patients were classified as nourished, suspected/moderately malnourished, or severely malnourished. For statistical purposes, nutritional status was

14
categorized into two groups: nourished and malnourished (suspected/moderate and severe). Anthropometric evaluation included measurement of the triceps skinfold thickness (TSF) with a Lange Skinfold Caliper (Cambridge Scientific Industries Inc., Cambridge, MD, USA), the arm circumference (AC), with an inextensible tape, and both were used to calculate the arm muscle area (AMA)[23]. The anthropometric measurements were classified according to Frisancho[24]. The measured values of TSF, AC and AMA below the 5th percentile were considered malnutrition [24]. Only one investigator performed the measurements to minimize practical variability, and the average of three consecutive measurements was recorded.

Muscle functional status was evaluated by handgrip dynamometry (Jamar Plus + ®) according to the established protocol by Budziareck et al.(2008) [25]. Assessments were performed in triplicate, and the average value was used ([25], with values below the 5th percentile considered to indicate malnutrition. The 6-minute walking test (6MWT) was performed according to the American Thoracic Society guidelines [26] .

Resting energy expenditure (REE) was measured by indirect calorimetry (IC) using the Quark RMR device (Cosmed, Rome, Italy). The test was performed in a silent, temperature-controlled room (22–24 °C) in the morning. The patients fasted for 12 h, and remained recumbent for approximately 20 min before beginning the test. The total energy expenditure (TEE) was calculated by adding 20% to the value of the REE ([27, 28].

Quantitative food intake data were obtained using the 24-hour recall (R24h). The 24-hour recall was collected according to the multiple pass method, whereby the interviewee is guided by five steps [29]. This method helps the individual remember the food and drink consumed on the day before the interview and to report them in detail,

reducing errors in dietary measurement. Diet Pro4R® software (Agromidia Software, Viçosa, Brazil) was used to calculate daily energy intake (EI), proteins, carbohydrates, and lipids. Protein intake was considered adequate for the minimal amounts of 1.2 g/kg of dry body weight [28]. Energy balance (EB) was calculated as the difference between EI obtained in the R24h and TEE (EB= EI – TEE); an EB below zero was considered negative.

The HRQoL was evaluated by the Chronic Liver Disease Questionnaire (CLDQ). This is a short instrument consisting of 29 questions distributed in six domains: abdominal symptoms (ABM), fatigue (FAM), systemic symptoms (SYM), activity (ACM), emotional function (EMM) and worry (WOM), and each domain has seven levels of responses: from 0 (all time) to 6 (never). The score in each domain was obtained by the sum of the answers and divided by the number of questions answered. These data were classified according to Souza et al. 2015[30]. Low HRQoL was designated when the total CLDQ score was <5, and high HRQoL when ≥5.

The study sample size was based on the study by Ong., et al., 2011[31] who showed patients with chronic liver disease had an improved of 1.51 points in the CLQD score after the intervention with branched-chain amino acids. Thus, our estimated sample size was 42 patients (21 patients for each group), considering a power of 80%, an alpha of 0.05 and 30.0% of loss of follow up.

Frequency distributions, measures of central tendency and dispersion were calculated, and the Shapiro-Wilk test was used to verify the normality of the quantitative variables. The variables of normal distribution were presented as mean and standard deviation, and the nonparametric data as median and interquartile range. The t Student and Wilcoxon tests were used for intra-group comparisons of means and medians, respectively, to evaluate the effectiveness of the intervention. Categorical data

were compared using the Chi-square test or Fisher's test when appropriate. A significance level of 5% was adopted for all analyses. The Stata Statistical Software, version 12.0 was used.

Results

Fifty six patients were recruited and randomized into the WP and C groups. Seven patients dropped out during treatment (Figure 1). There was no difference between the groups in terms of the general characteristics (Table.1) and nutritional status at baseline ($p>0.05$). According to SGA, 48.0% and 54.0% of patients were malnourished in the WP and C groups, respectively. There were no significant differences considering dynamometry (WP group: 29.53± 1.69 Kg; C group: 31.06±1.64 Kg; $p>0.05$) and the 6MWT (WP group: 446.0 ± 20.7 meters; C group: 454.6 ± 19.6 meters; $p>0.05$).

Energy (20.2 vs 23.0 kcal/kg) and protein (0.8 vs 0.9 g/kg) intake were similar between the groups before the protein supplementation ($p >0.05$), with 78.6% and 73.1% of the patients in the WP and C group, respectively, eating less than the recommended protein allowances. The mean EB was -558.7 ± 639.7 kcal in the WP group and -413.2 ± 504.4 kcal in the C group ($p>0.05$).

At the beginning of the study, the HRQoL of patients was similar between the groups. The total CLDQ score was 4.0 for the WP group and 3.8 for the C group ($p>0.05$), with only 10.3% of the patients presenting a total score over 5.0, prior to intervention. Only nutrition status was associated with poor HRQoL ($p<0.05$). Among those individuals who had HRQoL<5, 64.7% were classified as moderately or severely malnourished. Neither the severity of the disease (indicated by the MELD score) nor the functional status influenced the HRQoL of patients ($p>0.05$).

Patients in both groups presented an increased quality of life after the intervention. The CLDQ score increased by 0.97 points (3.73 ± 1.03 before the intervention and 4.70 ± 0.68 after the intervention) ($p < 0.05$). After the intervention, 47.8% of the patients presented a total score over 5.0. However, there were no significant differences between the groups ($p > 0.05$). Considering each CLDQ domain, most scores increased after the protein supplementation compared to the baseline ($p < 0.05$), except for the domains of abdominal symptoms ($p > 0.05$) (Figure 2A; B). The intra analyses of both groups revealed that the FAM domain represented the greatest difference ($p < 0.05$) (Table 3). Patients who had a positive EB together with adequate protein requirements exhibited a higher HRQoL score (4.9) than those who only ingested the required protein (4.3). No significant differences, between group, were observed ($p < 0.05$).

Throughout the study, protein intake among patients in the WP group increased to 1.4 ± 0.6 g/kg, similar to the C group ($p > 0.05$). Although most patients ingested the recommended allowance of protein, the majority of the patients still presented a negative EB (-424.5 ± 584.9 kcal for the WP, and -350.6 ± 607.3 kcal for the C group) ($p > 0.05$).

There were no changes in the anthropometric measures (AC and TSF) or in the handgrip strength after the supplementation ($p > 0.05$). The mean 6MWT was similar before and after supplementation ($p > 0.05$) (Table 2).

Discussion

The quality of life of patients with advanced liver disease awaiting transplantation improved after whey protein and casein supplementations. Protein

supplementation has been previously associated with an improved HRQoL in malnourished individuals with gastrointestinal diseases[32], chronic heart failure, and cachexia [33] as well as in sarcopenic elderly adults [34, 35].

To our knowledge, no previous study has evaluated the short-term effects of WP supplementation and the resulting HRQoL in cirrhotic patients waiting for liver transplantation. In this population, studies have been limited to branched-chain amino acid (BCAA) or nutritional counseling alone [36]. Kawamura et al., 2004[37] evaluated the effect of BCAA supplementation on the HRQoL of 25 patients with hepatic cirrhosis during 6 months. Patients in the BCAA group presented improved HRQoL when compared to those in the control group. According to the authors, this improvement in the HRQoL could be attributed to the decrease of secondary symptoms, such as edema and ascites. Muto et al. 2005 [38] found similarly improved results in a controlled study conducted with 646 patients with decompensated cirrhosis who were given BCAA (12 g/day) for 2 years versus a group with a standardized diet. Nonetheless, the comparison of our results to these studies is difficult and is further limited by the fact that none used the CLDQ to assess the HRQoL. The advantage of using this questionnaire over other generic questionnaires is its specificity for assessing liver disease. The CLDQ is the only instrument developed for all etiologies and degrees of severity in liver disease, and it was validated for the Brazilian population[39].

Poor HRQoL was associated with a deficient nutritional status, since 64.7% of those with scores <5 were classified as moderately or severely malnourished. Malnutrition in end-stage liver disease patients is multifactorial, a consequence of early satiety due to ascites and portal hypertension, appetite loss and poor nutrient intake (often related to unnecessary salt and protein restriction), as well as the use of various medications[40]. Most patients in our study had inadequate energy and protein intake. At

study baseline, only 16.0% and 21.4% of the WP patients received adequate energy and protein intake, respectively. We had previously reported in a study conducted with 73 pre-LT patients that more than 80% of our patients had insufficient energy intake [7]. In another study conducted by Ney et al., 2015 [41] 76.0% of the 630 pre-LT patients demonstrated a protein intake below 1.2 g / kg / day.

We hypothesized that WP supplementation, although for a short-time, would contribute to improving these patients' nutritional and functional status, due to its higher digestibility and the increased number of branched-chain amino acids, especially leucine, thereby improving their quality of life. However, we observed improvement only in the quality of life. These results are partially in accordance with those by Boulhosa et al[42] who reported the effect of 12 weeks of supplementation of two different proteins (casein and soy protein) on the HRQoL of patients with hepatitis C virus. Similar to our study, both groups presented an increased HRQoL score, measured by the Short Form Health Surveillance (SF-36) questionnaire. The authors showed that the improvement in HRQoL was attributed to an increase in lean mass over the 12 weeks period, which we did not observe with our patients. Certainly, the short time supplementation is the main factor impacting our nutritional and functional results. In most of the studies, the time of amino acid or protein supplementation was greater than 30 days[33, 34, 37, 38]. Also, in our study, although most of the patients reached the recommended amount of protein after supplementation, only 25.0% had a positive energy balance at the end. An adequate supply of both energy and protein is essential for protein synthesis[15], and nutritional and functional status improvement. Finally, it is also important to acknowledge that the instruments we used to assess the nutritional and functional status maybe incapable of diagnosing minor changes. In this regard, metabolic alterations are the first to be improved, followed by

functional capacity markers, and only later anthropometric values [25, 43]. On the other hand, it is possible that peptides derived from digestive processes may have exerted positive metabolic effects increasing the quality of life. The interaction of peptides with enteric nerves and endocrine gastrointestinal systems influence the metabolic and physiological fate of proteins[44]. Currently, there is an increase in research reporting positive effects on brain function and cognitive status associated with components derived from milk protein[45-47]. However, we did not see a difference between the two supplemented proteins, and this leads us to speculate the individualized attention and time spent explaining the study protocol and its potential benefits may have influenced measurements of the quality of life.

The impact of emotional factors on HRQoL was observed in a study conducted by Sharif et al. (2005) [48]. They evaluated the HRQoL of 100 patients waiting for LT randomly divided into two groups: control and psychoeducational intervention (information regarding liver disease adaptation to chronic diseases relaxation, exercise, diet and side effects of drugs). The results revealed significant differences in all domains of the CLDQ in the group that received the psychoeducational intervention, while there were no statistically significant differences in the control group[48].

Finally, our study has limitations. The short duration of protein supplementation was determined, after the pilot study was carried out, in which patients' compliance was low, after the second week. Second, the insensitivity of the tools used to detect changes in nutritional status in short periods is a reality of the current clinical available assessment methods. Furthermore, the use of a 24-hour recall to estimate the daily energy/protein intake may not accurately represent the daily intake throughout the study period, but once again, patients' adherence to daily registers was low.

Conclusion

Our results indicate that short-term whey protein and casein supplementation improve the HRQoL of patients waiting for LT.

References

1. ABTO, Dimensionamento dos Transplantes no Brasil e em cada estado (2007-2014). 2014: Registro Brasileiro de Transplantes.

2. Gad EH, Alsebaey A, Lotfy M, et al. Complications and mortality after adult to adult living donor liver transplantation: A retrospective cohort study. Ann Med Surg (Lond). 2015; 4(2): 162-71.

3. Kim WR, Lake JR, Smith JM, et al. OPTN/SRTR 2013 Annual Data Report: liver. Am J Transplant. 2015; 15 Suppl 2: 1-28.

4. Heidelbaugh JJ, Bruderly M. Cirrhosis and chronic liver failure: part I. Diagnosis and evaluation. Am Fam Physician. 2006; 74(5): 756-62.

5. Labenz C, Toenges G, Schattenberg JM, et al. Health-related quality of life in patients with compensated and decompensated liver cirrhosis. Eur J Intern Med. 2019; 70: 54-59.

6. Nishida Y, Ide Y, Okada M, et al. Effects of home-based exercise and branched-chain amino acid supplementation on aerobic capacity and glycemic control in patients with cirrhosis. Hepatol Res. 2017; 47(3): E193-E200.

7. Ferreira LG, Martins AI, Cunha CE, et al. Negative energy balance secondary to inadequate dietary intake of patients on the waiting list for liver transplantation. Nutrition. 2013; 29(10): 1252-8.

38
2998. Kalaitzakis E, Simren M, Olsson R, et al. Gastrointestinal symptoms in patients
300 with liver cirrhosis: associations with nutritional status and health-related quality
301 of life. Scand J Gastroenterol. 2006; 41(12): 1464-72.
3029. Les I, Doval E, Flavia M, et al. Quality of life in cirrhosis is related to
303 potentially treatable factors. Eur J Gastroenterol Hepatol. 2010; 22(2): 221-7.
30410. Poon RT, Yun WC, Fan ST, et al. Long-term oral branched chain amino acids in
305 patients undergoing chemoembolization for hepatocellular carcinoma: a
306 randomized trial. Aliment Pharmacol Ther. 2004; 19(7): 779-88.
30711. Norman K, Henriette K, Herbert L, et al. Malnutrition affects quality of life in
308 gastroenterology patients. World J Gastroenterol. 2006; 12(21): 3380-5.
30912. Qiao CX, Zhai XF, Ling CQ, et al. Health-related quality of life evaluated by
310 tumor node metastasis staging system in patients with hepatocellular carcinoma.
311 World J Gastroenterol. 2012; 18(21): 2689-94.
31213. Younossi ZM, Guyatt G, kiwi M, et al. Development of a disease specific
313 questionnaire to measure health related quality of life in patients with chronic
314 liver disease. Gut. 1999; 45(2): 295-300.
31514. Nakaya Y, Okita K, Moriwaki H, et al. BCAA-enriched snack improves
316 nutritional state of cirrhosis. Nutrition. 2007; 23(2): 113-120.
31715. Phillips SM. The impact of protein quality on the promotion of resistance
318 exercise-induced changes in muscle mass. Nutr Metab (Lond). 2016; 13: 64.
31916. Norton LE, Layman DK, Bunpo P, et al. The leucine content of a complete
320 meal directs peak activation but not duration of skeletal muscle protein synthesis
321 and mammalian target of rapamycin signaling in rats. J Nutr. 2009; 139(6):
322 1103-9.

41
32317. West DW, Burd NA, Coffey VG, et al. Rapid aminoacidemia enhances
324 myofibrillar protein synthesis and anabolic intramuscular signaling responses
325 after resistance exercise. Am J Clin Nutr. 2011; 94(3): 795-803.
32618. Sahathevan S, Se CH, Ng S, et al. Clinical efficacy and feasibility of whey
327 protein isolates supplementation in malnourished peritoneal dialysis patients: A
328 multicenter, parallel, open-label randomized controlled trial. Clin Nutr ESPEN.
329 2018; 25: 68-77.
33019. Devries MC, Phillips SM. Supplemental protein in support of muscle mass and
331 health: advantage whey. J Food Sci. 2015; 80 Suppl 1: A8-A15.
33220. Luiking YC, Deutz NE, Memelink RG, et al. Postprandial muscle protein
333 synthesis is higher after a high whey protein, leucine-enriched supplement than
334 after a dairy-like product in healthy older people: a randomized controlled trial.
335 Nutr J. 2014; 13: 9.
33621. Tosukhowong P, Boonla C, Dissayabutra T, et al. Biochemical and clinical
337 effects of Whey protein supplementation in Parkinson's disease: A pilot study. J
338 Neurol Sci. 2016; 367: 162-70.
33922. Detsky AS, McLaughlin JR, Baker JP, et al. What is subjective global
340 assessment of nutritional status? JPEN J Parenter Enteral Nutr. 1987; 11(1): 8-
341 13.
34223. Lohman T, Roche A, Martorell R. Anthropometric standardization reference
343 manual. 1988.
34424. Frisancho AR, Smith SL. Reduction in birth weight associated with smoking
345 among young and older-age women. Am J Hum Biol. 1990; 2(1): 85-88.

44
34625. Budziareck MB, Duarte RR, Barbosa-Silva MC. Reference values and 347 determinants for handgrip strength in healthy subjects. Clin Nutr. 2008; 27(3): 348 357-62.
34926. Laboratories, A.C.o.P.S.f.C.P.F., ATS statement: guidelines for the six-minute 350 walk test. Am J Respir Crit Care Med. 2002; 166(1): 111-7.
35127. easloffice@easloffice.eu, E.A.f.t.S.o.t.L.E.a. and E.A.f.t.S.o.t. Liver, EASL 352 Clinical Practice Guidelines on nutrition in chronic liver disease. J Hepatol. 353 2019; 70(1): 172-193.
35428. Plauth M, Bernal W, Dasarathy S, et al. ESPEN guideline on clinical nutrition in 355 liver disease. Clin Nutr. 201; 38(2): 485-521.
35629. Moshfegh AJ, Rhodes DG, Baer DJ, et al. The US Department of Agriculture 357 Automated Multiple-Pass Method reduces bias in the collection of energy 358 intakes. Am J Clin Nutr. 2008; 88(2): 324-32.
35930. Souza NP, Villar LM, Garbin AJ, et al. Assessment of health-related quality of 360 life and related factors in patients with chronic liver disease. Braz J Infect Dis. 361 2015; 19(6): 590-5.
36231. Ong JP, Oehler G, Jansen CK, et al. Oral L-ornithine-L-aspartate improves 363 health-related quality of life in cirrhotic patients with hepatic encephalopathy: an 364 open-label, prospective, multicentre observational study. Clin Drug Investig. 365 2011; 31(4): 213-20.
36632. Norman K, Smoliner C, Valentini L, et al. Is bioelectrical impedance vector 367 analysis of value in the elderly with malnutrition and impaired functionality? 368 Nutrition. 2007; 23(7-8): 564-9.
36933. Rozentryt P, Haehling SV, Lainscak M, et al. The effects of a high-caloric 370 protein-rich oral nutritional supplement in patients with chronic heart failure and

cachexia on quality of life, body composition, and inflammation markers: a randomized, double-blind pilot study. J Cachexia Sarcopenia Muscle. 2010; 1(1): 35-42.

34. Rondanelli M, Klersy C, Terracol G, et al. Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. Am J Clin Nutr. 2016; 103(3): 830-40.

35. Abizanda P, Atienzar P, Casado L, et al. Cardiovascular risk factors are associated with subclinical atherosclerosis in high functioning older adults. Maturitas. 2010; 67(1): 54-9.

36. Krasnoff JB, Vintro AQ, Ascher NL, et al. A randomized trial of exercise and dietary counseling after liver transplantation. Am J Transplant. 2006; 6(8): 1896-905.

37. Kawamura N, Nakajima H, Takashi SI. Administration of granulated BCAA and quality of life. Hepatol Res. 2004; 30S: 42-45.

38. Muto Y, Sato S, Watanabe A, et al. Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol. 2005; 3(7): 705-13.

39. Mucci S, Citero VA, Gonzalez AM, et al. [Cross-cultural adaptation of the Chronic Liver Disease Questionnaire (CLDQ) to the Brazilian population]. Cad Saude Publica. 2010; 26(1): 199-205.

40. Fábregas BC, Ávila RE, Faria MN, et al. Health related quality of life among patients with chronic hepatitis C: a cross-sectional study of sociodemographic, psychopathological and psychiatric determinants. Braz J Infect Dis. 2013; 17(6): 633-9.

41. Ney M, Abraldes JG, Ma M, et al. Insufficient Protein Intake Is Associated With Increased Mortality in 630 Patients With Cirrhosis Awaiting Liver Transplantation. Nutr Clin Pract. 2015; 30(4): 530-6.

42. Boulhosa RS, Oliveira LP, Jesus RP, et al. The impact of nutritional supplementation on quality of life in patients infected with hepatitis C virus. J Hum Nutr Diet. 2013; 26 Suppl 1: 7-15.

43. Berry JK, Braunschweig CA. Nutritional assessment of the critically ill patient. Crit Care Nurs Q. 1998; 21(3): 33-46.

44. Jahan-Mihan A, Luhovyy BL, Khoury DE, et al. Dietary proteins as determinants of metabolic and physiologic functions of the gastrointestinal tract. Nutrients. 2011; 3(5): 574-603.

45. Camfield DA, Owen L, Scholey AB, et al. Dairy constituents and neurocognitive health in ageing. Br J Nutr. 2011; 106(2): 159-74.

46. Crichton GE, Elias MF, Dore GA, et al. Relation between dairy food intake and cognitive function: The Maine-Syracuse Longitudinal Study. Int Dairy J. 2012; 22(1): 15-23.

47. Markus CR, Olivier B, de Haan EH. Whey protein rich in alpha-lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects. Am J Clin Nutr. 2002; 75(6): 1051-6.

48. Sharif F, Mohebbi S, Tabatabaee HR, et al. Effects of psycho-educational intervention on health-related quality of life (QOL) of patients with chronic liver disease referring to Shiraz University of Medical Sciences. Health Qual Life Outcomes. 2005; 3: 81.

Figure Legends

Figure 1. Flowchart of the participants during the phases of recruitment, randomization and intervention.

Abbreviation: WP: whey protein group; C: casein group

Figure 2. Mean score of CLDQ domains in the WP (A) and C (B) groups comparing pre and post supplementation.

Abbreviation: WP: whey protein group; C: casein group; ABM: Abdominal symptoms; FAM: Fatigue SYM: Systemic symptoms; ACM: domain activity; EMM: Emotional function; WOM: Worry

Table 1. Characterization of the patients at baseline.

Characteristics	WP (n=25)	C (n=24)	P
Age (years)	53.3±10.2	50. 4±12.4	>0.05 γ
CLD etiology (n,%)			
Ethanolic	8.0 (32.0%)	5.0 (20.8%)	> 0.05 ɬ
Virus B and C	10.0 (40.0%)	12.0 (50.0%)	>0.05 ɬ
Other	7.0 (28.0%)	7.0 (29.2%)	>0.05 ɬ

59

MELD	15.3 ± 0.7	15.6 ± 0.8	>0.05*
Edema (n,%)	16.0 (64.0%)	16.0 (66.6%)	>0.05 ł
Ascites (n, %)	12.0 (54.5%)	18.0 (75.0%)	>0.05 ł
Medications ≥3 (n,%)	21.0 (84.0%)	21.0 (87.5%)	>0.05 ł

455* t test; γ Wilcoxon; ł chi-square

456Abbreviations: WP: whey protein group; C: casein group; CLD:Chronic Liver Disease;

457MELD:Model for End-Stage Liver Disease

458

459

460

462

463

464

465

466

467**Table 2. Nutritional and functional status before and after protein supplementation.**

Group	Parameters	Before (mean ± SD)	After (mean ± SD)	P
WP	AC	30.4 ± 0.9	30.1± 1.0	NS
C	AC	28.6 ±4.2	29.7 ± 1 .0	NS
WP	Dynamometry	29.53± 1.69	30.68± 1.8	NS

C	Dynamometry	31.06 ±1.64	31.18 ± 2.5	NS
WP	TSF	21.04± 10.39	20.62 ± 9.9	NS
C	TSF	18.10 ± 10.31	19.49 ± 10.4	NS
WP	6MWT	446.0m±20.75	454.61±19.6	NS
C	6MWT	433.5m4±21.35	449.62m ±16.6	NS

Abbreviations: WP: whey protein group; C: casein group**;** AC: arm circumference; TSF: triceps skinfold thickness; 6MWT :6-minute walking test

Table 3. Results of CLDQ by domains before and after protein supplementation.

Group	Domains	Mean	Delta	P
WP	Pre ABM	4.3	1.0	p>0.05
	Post ABM	5.3		

65

	Pre FAM	3.5	1.3	p< 0.05
	Post FAM	4.8		
	Pre SYM	3.8	1.0	p< 0.05
	Post SYM	4.8		
	Pre ACM	5.0	1.0	p< 0.05
	Post ACM	6.0		
	Pre EMM	3.4	0.6	p< 0.05
	Post EMM	4.0		
	Pre WOM	4.0	1.0	p< 0.05
	Post WOM	5.0		
	Pre TOTAL	4.0	1.0	p< 0.05
	Post TOTAL	5.0		
Group	**Domains**	**Medium**		**p value**
C	Pre ABM	4.0	1.3	p< 0.05
	Post ABM	5.3		
	Pre FAM	3.4	1.6	p< 0.05
	Post FAM	5.0		
	Pre SYM	4.0	0.8	p< 0.05
	Post SYM	4.8		
	Pre ACM	4.1	1.5	p< 0.05
	Post ACM	5.6		
	Pre EMM	3.0	0.9	p< 0.05
	Post EMM	4.0		
	Pre WOM	3.7	1.2	p=0.115
	Post WOM	4.9		
	Pre TOTAL	3.8	1.1	p< 0.05
	Post TOTAL	4.9		

Abbreviations: WP: whey protein group; C: casein group; Pre: before the supplementation; Post: after

68
supplementation; ABM: Abdominal symptoms; FAM: Fatigue SYM: Systemic symptoms; ACM: domain activity; EMM: Emotional function; WOM: Worry.

471

69