UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

LAÍS AUGUSTI

Qual dos aminoácidos de cadeia ramificada aumenta o fluxo cerebral na encefalopatia hepática? Ensaio clínico randomizado e duplo-cego

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Doutora em Fisiopatologia em Clínica Médica.

Orientador: Prof. Dr. Fernando Gomes Romeiro

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DEDICATÓRIA

Aos meus pais, **Augusti** e **Lourdes**, pela liberdade, oportunidade e suporte de sempre. Não importa onde eu esteja, o que faça e como seja, a razão do meu empenho será sempre vocês. Amo vocês!

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por ter despertado em mim o interesse pela pesquisa e por ter me mostrado que as fronteiras da Nutrição eram maiores do que imaginava. Este trabalho é fruto da admiração pela ciência que você alimentou em mim e nos demais alunos que tiveram o privilégio de aprender com você em sua breve, porém fundamental, jornada.

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RESUMO

Romeiro F.G., Ietsugu M.V., Franzoni L.C., Augusti L., Alvarez M, Santos L.A.A., Lima T.B., Koga K.H., Moriguchi S.M., Caramori C.A., Silva G.F., Betting L.E. **Which of the branched-chain amino acids increases cerebral blood flow in hepatic encephalopathy? A double-blind randomized trial.** *NeuroImage: Clinical.* 2018; 19:302–310.

(Abstract traduzido)

Aminoácidos de cadeia ramificada aumentam a perfusão cerebral de pacientes com encefalopatia hepática (EH), mas o aminoácido responsável por esse aumento e os mecanismos envolvidos ainda não são conhecidos. Este estudo comparou a perfusão cerebral e a melhora clínica durante a suplementação de leucina ou isoleucina. Após a randomização, 27 indivíduos com cirrose e EH receberam suplementos de leucina ou isoleucina por um ano. Exames de tomografia computadorizada por emissão de fóton único (SPECT) e cintilografia cerebral dinâmica (DBS) foram realizados antes do estudo e após 1, 8 e 12 meses de suplementação. Apenas o grupo que recebeu isoleucina teve aumento da perfusão cerebral aos 8 meses de tratamento pelo exame de SPECT e pela cintilografia ($p<0,001$ e $p = 0,05$, respectivamente), também observado pelo SPECT aos 12 meses de suplementação ($p < 0,05$). O aumento do fluxo cerebral foi associado a melhora da EH aos 8 e 12 meses de suplementação ($p=0,008$ e $0,004$, respectivamente), porém essa melhora não foi observada no grupo que recebeu leucina ($p=0,313$ e $0,055$, respectivamente). A suplementação com isoleucina obteve melhor impacto na restauração da perfusão cerebral em pacientes com EH.

Palavras chave: Encefalopatia Hepática, Cirrose Hepática, Aminoácidos de cadeia ramificada, Perfusão cerebral.

ABSTRACT

Romeiro F.G., Ietsugu M.V., Franzoni L.C., Augusti L., Alvarez M, Santos L.A.A., Lima T.B., Koga K.H., Moriguchi S.M., Caramori C.A., Silva G.F., Betting L.E. **Which of the branched-chain amino acids increases cerebral blood flow in hepatic encephalopathy? A double-blind randomized trial.** *NeuroImage: Clinical.* 2018; 19:302–310.

Branched-chain amino acids increase the brain perfusion of patients with hepatic encephalopathy (HE), but the amino acid and the mechanisms involved are still unknown. This study compared brain perfusion and clinical improvement during leucine or isoleucine supplementation. After randomization, 27 subjects with cirrhosis and HE received leucine or isoleucine supplements for one year. Brain single photon emission computed tomography (SPECT) and dynamic brain scintigraphy (DBS) were performed pretreatment and at 1, 8 and 12 months of supplementation. Brain perfusion was increased only in the isoleucine group at 8 months of treatment by both SPECT and DBS ($p<0.001$ and $p = 0.05$, respectively) and by SPECT at the 12^{th} month ($p < 0.05$). This was associated with hepatic encephalopathy improvement at 8 and 12 months ($p=0.008$ and 0.004, respectively), which was not observed in the leucine group ($p=0.313$ and 0.055, respectively). Isoleucine supplementation achieved a better impact on brain perfusion restoration in HE.

Keywords: Hepatic encephalopathy, Liver cirrhosis, Branched-chain amino acids, Cerebral blood flow.

SUMÁRIO

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Which of the branched-chain amino acids increases cerebral blood flow in hepatic encephalopathy? A double-blind randomized trial

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ABSTRACT

Branched-chain amino acids increase the brain perfusion of patients with hepatic encephalopathy (HE), but the amino acid and the mechanisms involved are still unknown. This study compared brain perfusion and clinical improvement during leucine or isoleucine supplementation. After randomization, 27 subjects with cirrhosis and HE received leucine or isoleucine supplements for one year. Brain single Photon Emission Computed Tomography (SPECT) and dynamic brain scintigraphy (DBS) were performed pretreatment and at 1, 8 and 12 months of supplementation. Brain perfusion was increased only in the isoleucine group at 8 months of treatment by both SPECT and DBS ($p < 0.001$ and $p = 0.05$, respectively) and by SPECT at the 12th month ($p < 0.05$). This was associated with hepatic encephalopathy improvement at 8 and 12 months ($p = 0.008$ and 0.004, respectively), which was not observed in the leucine group ($p = 0.313$ and 0.055, respectively). Isoleucine supplementation achieved a better impact on brain perfusion restoration in HE.

1. Introduction

Hepatic encephalopathy (HE) has a harmful impact on chronic and acute liver illnesses and affects more than one third of patients with liver cirrhosis. After overt episodes, the one-year mortality of these patients varies from 42 to 64%, which is even worse than the values attributed to other complications (Bustamante et al., 1999; Jepsen et al., 2010; Stewart et al., 2007). Hyperammonemia is the chief disturbance in HE physiopathology, when the conversion of ammonia to glutamine (GLN) is fully activated in extrahepatic tissues, thus increasing the influx of intracellular water into astrocytes due to GLN osmoregulation (Brusilow et al., 2010). Furthermore, the high permeability of the blood-brain barrier facilitates the influx of ammonia to the brain under inflammatory conditions (Alonso et al., 2014). For practical purposes, in this article the word ammonia is used to represent ammonia free base (NH3) plus ammonium ($NH4+$).

HE treatment is based on controlling the trigger factors and managing ammonia production and absorption from the gut, by using disaccharides or antibiotics. Since many patients do not achieve sufficient improvement, branched-chain amino acids (BCAA) and drugs aiming to

Abbreviations: AC, arm circumference; APMT, adductor pollicis muscle thickness; BCAA, branched-chain amino acids; BCKA, branched-chain ketoacids; BMI, body mass index; CAMA, corrected mid-arm muscle area; CBF, cerebral blood flow; EEG, electroencephalogram; FDR, false discovery rate; GDH, glutamate dehydrogenase; GLN, glutamine; GLU, glutamate; HE, hepatic encephalopathy; HGS, handgrip strength; HPLC, high-performance liquid chromatography; HRQoL, health-related quality of life; MAMC, mid-arm muscle circumference; MELD, Model of End-Stage Liver Disease; NH3, ammonia; PDH, pyruvate dehydrogenase complex; ROIs, regions of interest; ROS, reactive oxygen species; SF-36, 36-item Short-Form General Health Survey; SPECT, Single Photon Emission Computed Tomography; SPM12, Statistical Parametrical Mapping 12; TCA, tricarboxylic acid; TSF, triceps skinfold; α-KG, α-ketoglutarate; αKGDH, α-ketoglutarate dehydrogenase complex

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increase ammonia conversion to urea are often added as adjuvant therapies.

Among all these options, BCAA (leucine, isoleucine and valine) have the least understood mechanism of action. The efficacy of these substances in HE treatment is well established from systematic reviews and meta-analyses (Gluud et al., 2015). Even so, the amount of BCAA and the proportion of each amino acid that patients should receive is still a matter of controversy, because all clinical trials until now used them together, making it impossible to analyze the influence of each one on HE manifestations. Of note, BCAA are not only linked to HE improvement but also to increased cerebral perfusion (Iwasa et al., 2003).

The key disturbance in aminoacidemia related to HE is ammonia accumulation, which is present in > 80% of patients according to the diagnostic methods adopted (Al Sibae and McGuire, 2009). The main ammonia source in cirrhotic patients is the intestinal catabolism of GLN from the blood, in addition to a lesser content produced by intestinal bacteria (Olde Damink et al., 2002; Weber Jr and Veach, 1979). In normal adults, the daily amount produced is approximately 1000 mmol (Walker, 2014). When the conversion of ammonia to urea is insufficient, hyperammonemia increases GLN production in skeletal muscles and the brain (Lockwood et al., 1979). Since most patients with cirrhosis present moderate to severe malnutrition and loss of muscle mass, the high levels of circulating ammonia will increase glutamine production in the brain, thereby worsening HE manifestations (Merli et al., 2013; Romeiro and Augusti, 2015).

The chief extrahepatic detoxifying process in mammals is the activity of glutamine synthetase (Cooper, 2012), which begins with the conversion of glutamate (GLU) and ammonia into GLN (Eq. (1)). This reaction requires GLU provided from BCAA catabolism in skeletal muscles, where BCAA and α-ketoglutarate (α-KG) can be converted to GLU and branched-chain ketoacids (Eq. (2)) (Holecek, 2015).

$$GLU + NH3 + ATP \rightleftarrows GLN + ADP + Pi \quad (1)$$

$$BCAA + \alpha - KG \rightleftarrows GLU + BCKA \quad (2)$$

Hyperammonemia decreases extracellular BCAA and increases the release of branched-chain ketoacids from the muscle (Holecek et al., 2011). The reversible nature of these reactions makes the concentrations of substrates and products the main regulators of these processes (Holecek et al., 2011). Consequently, the patients would have high levels of ammonia, GLN and branched-chain ketoacids, but lower levels of BCAA in the extracellular fluid.

BCAA deficiency has a large impact on the tricarboxylic acid (TCA) cycle, because leucine and valine are involved in the generation of Acetyl-CoA and Succinyl-CoA, respectively, whereas isoleucine is the only amino acid that generates both of these products (Fig. 1). Therefore, the BCAA deficiency can lead to an impaired TCA cycle.

The effects of BCAA in the context of HE have been extensively studied (Bak et al., 2013; Holecek, 2015; Les et al., 2011; Yamamoto et al., 2005). Nevertheless, there is no standardization as to BCAA doses. In general, leucine, isoleucine and valine are administered together and in different proportions (Les et al., 2011; Marchesini et al., 2003). As a result, the appropriate amount of each amino acid to be prescribed is still unknown, but leucine and isoleucine are important to the oxidative metabolism of astrocytes (Bak et al., 2013; Johansen et al., 2007; Murin et al., 2009; Nissen et al., 2015).

The present study hypothesizes that the long-term effects of isoleucine leads to a higher increase in cerebral perfusion than that obtained by leucine, thus promoting different degrees of recovery from HE according to the amino acid received by the subjects. Hence, the study was focused on brain areas that are known for being more affected by hypoperfusion, such as the basal ganglia (Bizzi et al., 1996; Kumar et al., 1991; Sims and Pulsinelli, 1987), as well as regions where altered perfusion was already reported in HE and/or acquired hepatocerebral degeneration, such as the hippocampus, prefrontal cortex, medial temporal cortex, anterior cingulate cortex and parietooccipital regions (Lockwood et al., 1993; O'Carroll et al., 1991; Sunil et al., 2012; Ueki et al., 2002; Zafiris et al., 2004). Perfusion alterations in some of these regions were already associated with HE symptoms in prior studies, while some areas were reported as having dissimilar blood flow responses after BCAA administration (Catafau et al., 2000; Iwasa et al., 2003; Lockwood et al., 1993; O'Carroll et al., 1991; Ueki et al., 2002). Therefore, the primary aim of this study was to evaluate the effects of leucine and isoleucine supplementation on cerebral perfusion in patients with persistent HE. Secondary aims were to assess HE grade, body composition measurements and quality of life. The assessments were performed at 1, 8 and 12 months of treatment, and the serum amino acid levels were measured before and after the trial.

2. Materials and methods

Patients aged > 18 years with cirrhosis and persistent HE who attended the Hepatology units at UNESP Hospital (Botucatu, São Paulo state, Brazil) from 2014 to 2015 were invited to participate. Patients with other neurological diseases, hepatocellular carcinoma, prior liver transplantation or acute-on-chronic liver failure were excluded, as well as those who were already taking BCAA or had started/changed medications for HE treatment in the last week before entering the trial. Thus, only patients taking the same medications and doses for at least one week were included. The study was approved by the Comitê de Ética em Pesquisa (protocol number 4334/2012 at 09/03/2012) and received the Brazilian trial registration number RBR-8t4bf3. The study protocol was conducted according to the Declaration of Helsinki and its revisions. All the subjects or their caregivers signed the informed consent. When the patient was not able to sign, the signature of a family member was obtained.

Liver cirrhosis was established from a liver biopsy or from clinical and complementary exams. Clinical and nutritional evaluations were carried out before the supplementation and bimonthly afterwards, including anthropometric evaluation and handgrip strength measures.

HE was graded according to the West Haven criteria proposed by Amodio et al. and endorsed by the American and European Guidelines (Amodio et al., 2004; Vilstrup et al., 2014a, 2014b). Grade 0 comprised subjects without clinical signals of HE, in whom minimal HE was diagnosed according to specific exams (electroencephalogram). Grades I and II were categorized according to the current Guidelines (Vilstrup et al., 2014a, 2014b), thus classifying each neurological finding into a specific form. Since only outpatients were recruited, those with HE Grades III and IV were not included.

2.1. Sample size calculation and randomization plan

In a similar study, the brain perfusion standard deviation in the parietal lobe was 14% and increased by 20% after BCAA administration (Iwasa et al., 2003). Considering these same values, with respective alpha and beta errors of 0.05 and 0.2, the sample size required to find significant differences between the groups would be 16 subjects.

The subjects were randomized to participate in one of two groups. Additional information is included as Supplementary material. A control group under placebo treatment was not included due to ethical concerns, in agreement with prior recommendations (Als-Nielsen et al., 2004; Romeiro et al., 2013). The flow diagram of the patients included is shown in Fig. 2.

Each packet contained 5.0 g of maltodextrin, 1.8 g of maltitol and 1.2 g of flavoring plus 10.0 g of the amino acid, which was L-leucine or L-isoleucine according to the subjects' randomization (Ajinomoto® and Basecol Mix Indústria e Comércio de Alimentos LTDA, Brazil).

After starting the supplementation, the suggested schedule was to take 3 packets daily mixed in 200 mL of juice or dairy drinks ingested during breakfast, lunch and an evening snack for 12 months. BCAA doses of 30 g were previously given daily to HE patients by Les et al. (2011). Each subject was reevaluated every other month, when the

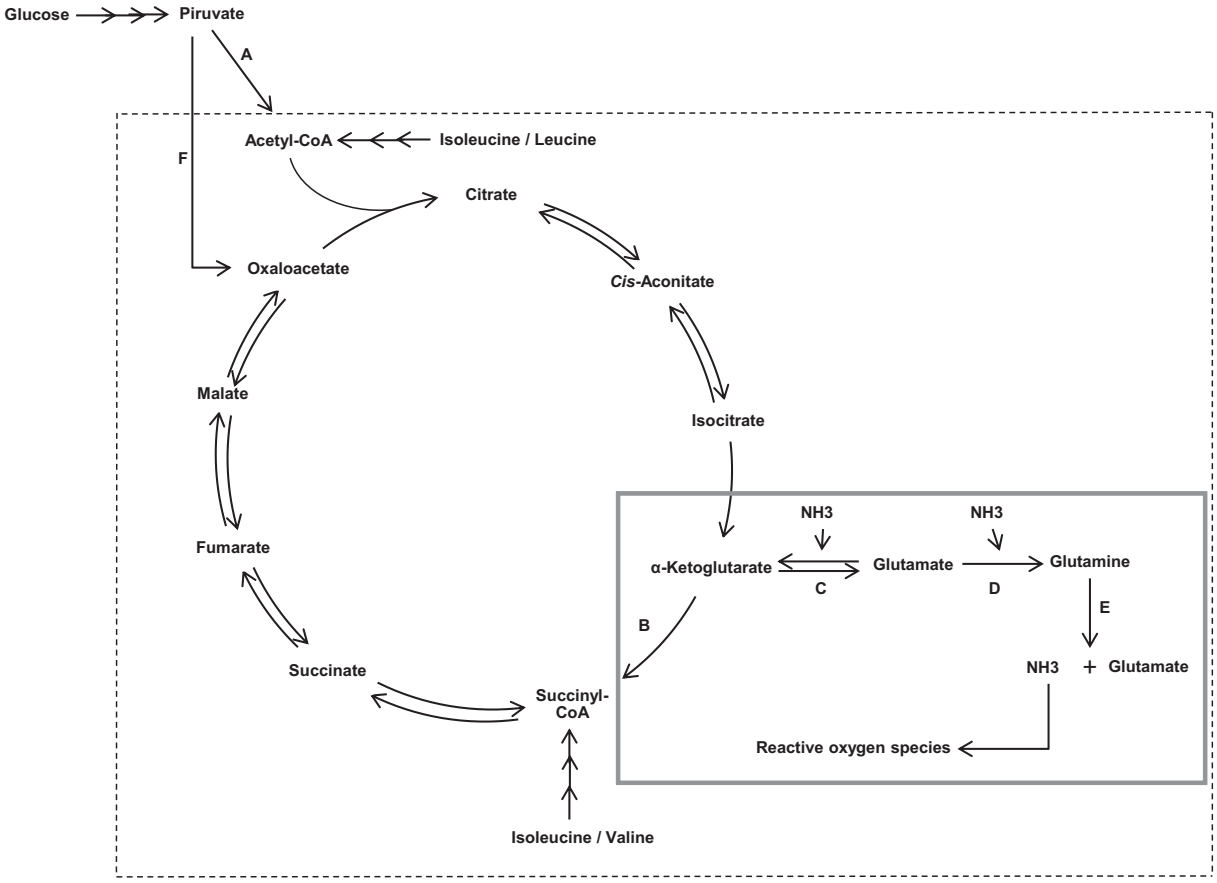

Fig. 1. Schematic representation of relevant metabolic reactions related to hyperammonemia and branched-chain amino acids. A = Pyruvate dehydrogenase, B = α-ketoglutarate dehydrogenase, C = glutamate dehydrogenase (the predominant reaction is the formation of α-ketoglutarate), D = glutamine synthetase, E = phosphate-activated glutaminase, F = pyruvate carboxylase, (→→→) = multiple steps, (————) = mitochondrial membrane. Some of the most important steps related to this study are shown in the gray rectangle.

adherence to treatment and the remaining packets were checked. Monthly phone calls were made to assure that the subjects were taking the supplement according to the trial schedule.

Before the amino-acid supplementation, the subjects were submitted to clinical exams, anthropometric assessment, handgrip strength measures, brain SPECT, dynamic brain scintigraphy, electroencephalogram and lab tests. The clinical exam was focused on grading HE and ascites. Laboratory exams included venous ammonia and the tests needed to calculate both the Model of End Stage Liver Disease score and the Child-Pugh classification.

2.2. Electroencephalogram

Electroencephalogram exams were used to diagnose minimal hepatic encephalopathy. Acquisition information is included as Supplementary Material.

2.3. Single Photon Emission Computed Tomography (SPECT)

The subject was kept in a supine position and free from audiovisual stimuli for at least 20 min before performing the brain SPECT acquisitions. Caffeine, alcohol and stimulants were not allowed starting on the day before the exam. Each patient received 1110 MBq (30 mCi) of 99mTc-bicisate ethyl cysteinate dimer through a peripheral vein one hour before the acquisition. A dual-headed gamma camera (Millennium MG system, General Electric, UK) was employed to acquire the images.

The photopeak was centered at 140 keV with 20% energy using low-energy and high-resolution collimators. The acquisition was made in a 128 × 128 matrix with voxel sizes of 2.26 × 2.26 × 2.26 mm^3, thus resulting in a resolution of 0.44 pixels/mm over 360° rotation performing 65 frames of 25 s each. A fifth-order Butterworth high-pass filter with cutoff frequency of 0.5 cycles/min was applied to perform the filtered backprojection reconstruction (Morano and Seibyl, 2003; Sunil et al., 2012). A specialist who was unaware of the supplement given performed the SPECT analysis (details included as Supplementary material).

2.4. Dynamic brain scintigraphy

Dynamic brain scintigraphy was also acquired with the patients in a supine position. Immediately before the acquisition, the subjects received 1110 MBq (30 mCi) of 99mTc through a peripheral vein. Images including head, neck and proximal thorax were acquired by a dual-headed gamma camera (Millennium MG system, General Electric, United Kingdom) equipped with low-energy high-resolution photopeak centered at 140 keV and 20% energy. Acquisition information is included as Supplementary material.

2.5. Nutritional assessment and quality-of-life survey

Nutritional status was evaluated through anthropometric measures and handgrip strength. Quality of life was assessed using the Medical Outcomes Study 36-item Short-Form General Health Survey (SF-36) validated for Portuguese. Additional information is included as Supplementary material.

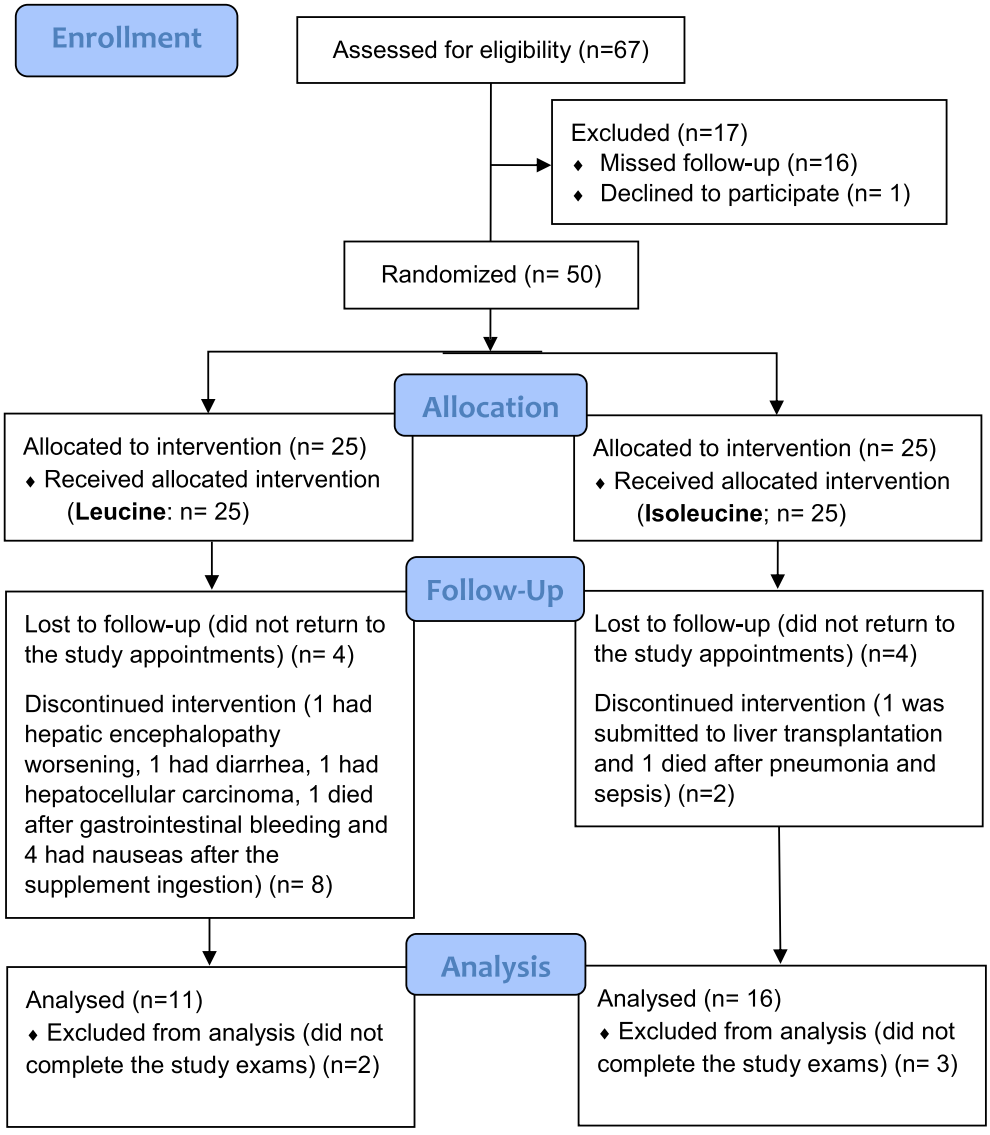

Fig. 2. Flow diagram of the subjects.

2.6. Amino-acid dosages

Serum levels of leucine and isoleucine from the subjects before and after the trial were obtained by high-performance liquid chromatography (SCL-10Avp control system, RF-10AXL fluorescence detector, Shimadzu, Japan). Chromatographic separation was achieved in a Shim-pack ISC-07/S1504 Na type sulfone group analysis column (4 mm × 150 mm i.d., 7 μm, Shimadzu, JPN), which was operated at 25 °C. A Shim-pack ISC-30/S0504 ammonia trap column was also used (4 mm × 50 mm i.d.) for suppressing baseline fluctuations that are often experienced in amino acid analysis. Additional information is included as Supplementary material.

2.7. Statistical analysis

Descriptive analysis was presented as median and interquartile ranges. Pretreatment comparisons between the groups were assessed by Mann-Whitney *U* test, whereas the individual results throughout the trial were evaluated by the Wilcoxon U test. The software Sigma Stat 3.5 (Systat Software Inc., San Jose, CA, USA) for Windows was used for the analysis.

Brain perfusion was assessed via the software SPM 12. The paired *t*-test was used to compare acquisitions at 1, 8 and 12 months of treatment with those before the supplementation. The anatomic visualization of the brain perfusion results from all patients of each group was obtained by a *t* statistic image, which was constructed and projected onto a standard high-resolution T1-weighted Magnetic Resonance Image (Song et al., 2014). The statistical significance level was defined as $p < 0.05$ for all the assays. For the SPECT comparisons throughout time, the *p* value was corrected using the false discovery rate (FDR), and some additional analyses were performed with $p < 0.001$.

3. Results

The subjects' initial characteristics did not differ between the groups. The number of male/female subjects was 8/3 in the leucine group and 12/4 in the isoleucine group ($p = 0.152$). The mean age was 61 years (52.5–65.7) in the leucine group and 57 years (52.0–62.0) in the isoleucine group ($p = 0.767$). Child-Pugh and MELD scores in the leucine and isoleucine groups were 8.00 (6.00–10.0) and 12.0 (8.50–15.5) in the former and 7.0 (6.5–8.5) and 11.0 (9.5–12.5) in the latter group, respectively ($p = 0.218$ and 0.535). The HE grade was 1 (0–1) in both groups ($p = 0.909$). Seven subjects in the leucine group and nine in the isoleucine group had minimal HE. Four subjects in the

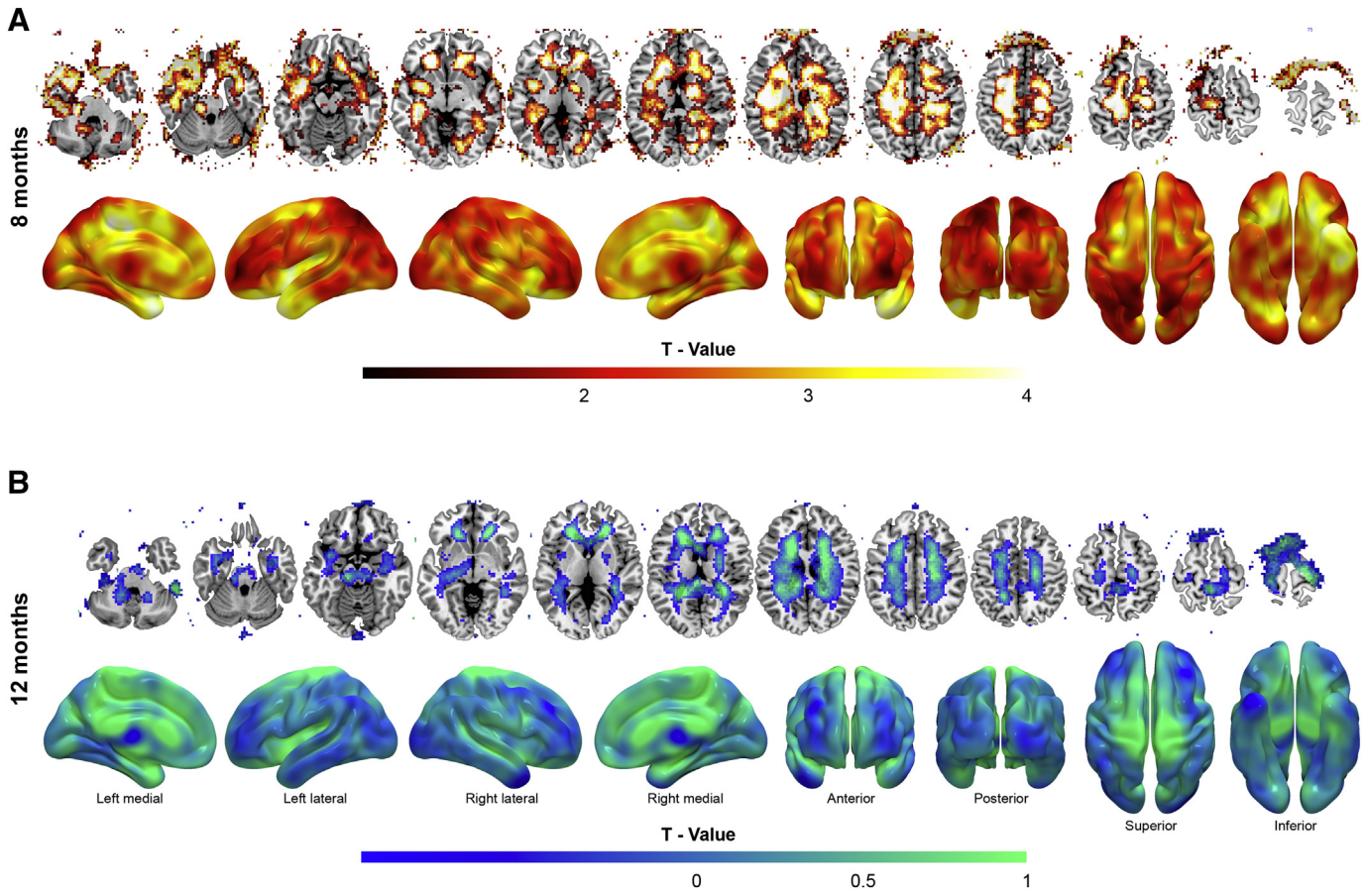

Fig. 3. Voxel-wise paired *t*-test comparison of cerebral perfusion between baseline and 8 months (A) and between baseline and 12 months (B) of isoleucine supplementation. Differences observed by brain SPECT are shown as color-coded regions in a total of 346 mm^3 for 8 months (hot color scale, threshold $p < 0.001$ uncorrected) and 48 mm^3 for 12 months (cold color scale, threshold $p < 0.05$ uncorrected). In the first row, results are overlaid in an anatomical MRI rendered in the axial orientation from inferior to superior and neurological orientation (right on right). In the second row, the results are overlaid in a tridimensional rendered model of an inflated brain (orientations are described below the renderings in panel B).

leucine group and six in the isoleucine group presented Grade I HE. One subject in the isoleucine group had Grade II HE.

In the SPECT analysis, there were no differences in cerebral perfusion after 1 month of treatment; the results obtained at 8 and 12 months of treatment were not significantly different from the baseline for patients receiving leucine supplements (data not shown). In contrast, a significant increase in cerebral perfusion was registered in the isoleucine group at 8 months of supplementation ($p < 0.001$) (Fig. 3A).

The main clusters of increased perfusion at 8 months were localized at: the right temporal lobe/inferior temporal gyrus (516 mm^3, p FDR corrected = 0.028, T value = 6.78, maximal value at x = 63 y = − 40 z = −34 Montreal Neurological Institute coordinates); right cerebral white matter mainly involving the pre-central gyrus region (1356 mm^3, p FDR corrected < 0.0001, T value = 5.46, x = 21 y = −19 z = 50); left cerebral white matter primarily involving the supplementary motor cortex, superior frontal gyrus, pre-central gyrus, middle cingulate gyrus and pre-central gyrus medial segment (8265 mm^3, p FDR corrected < 0.0001, T value = 3.80, x = −18 y = −13 z = 47); deep right cerebral white matter mainly involving the caudate, putamen, thalamus and pallidum (2181 mm3, p FDR corrected < 0.0001, T value = 3.52, x = 21 y = −4 z = 14).

This significant effect of isoleucine was also documented at 12 months of treatment. The difference at this time was less prominent when compared to the previous results, as displayed in Fig. 3B ($p = 0.05$). The main clusters of increased perfusion were localized at: the left cerebral white matter primarily involving the caudate and anterior cingulate gyrus (1119 mm^3, p uncorrected = 0.006, T value = 2.49, maximal value at x = −18 y = 20 z = 20), right cerebral white matter (1983 mm^3, p uncorrected = 0.007, T value = 2.78, x = 21 y = 35 z = 2) and left cerebral posterior white matter mainly involving the thalamus, middle and posterior cingulate gyrus (744 mm^3, p uncorrected = 0.009, T value = 2.35, x = −21 y = −40 z = 17).

To analyze the brain scintigraphy, the slope of the ascending curves of carotids and brain hemispheres were measured in order to quantify the cerebral blood flow. Again, the results obtained at the first month did not differ from those before the trial, and the results obtained at 8 and 12 months of treatment were not significantly different from the baseline for patients receiving leucine supplements (data not shown). In contrast, a clear enhancement of cerebral blood flow at 8 months of supplementation was observed in the isoleucine group, which was shown by the increase of slope curves of both hemispheres. However, it did not reach the significance level at the 12th month (Table 1).

The clinical evaluation findings at 8 and 12 months are described in Table 2. There were no significant changes in the Model of End Stage Liver Disease score during the trial. Reductions in Child-Pugh classification and HE grade did not achieve the significance level in the leucine group. In contrast, both Child-Pugh classification points and HE grade decreased at 8 and 12 months in the isoleucine group. Of note, ammonia levels did not change significantly during the trial.

Body assessment measures are shown in Table 3. For most nutritional variables, there were no significant differences between pre-treatment values and those measured at the 8th and 12th months, whereas triceps skinfold and handgrip strength increased significantly

Table 1
Dynamic brain scintigraphy analyses comparing the slope of the ascending curves obtained from carotids and brain hemispheres. The comparisons were performed between pretreatment values and the results at 8 and 12 months of supplementation.

		Amino acid					
		Leucine group (n = 11)			Isoleucine group (n = 16)		
		Pretreatment	8 months	12 months	Pretreatment	8 months	12 months
CA	R	72.8 (67.5–73.4)	76.6 (61.1–79.2)	69.4 (57.4–73.5)	72.0 (62.9–77.0)	75.5 (71.2–81.5)	71.9 (60.2–79.6)
	L	71.2 (58.7–78.8)	72.8 (58.5–75.9)	63.7 (59.9–73.5)	66.5 (54.6–75.2)	70.8 (57.7–77.9)	71.5 (55.9–80.1)
BH	R	59.9 (41.1–65.3)	60.9 (46.5–63.8)	52.8 (39.8–57.7)	50.2 (38.8–61.6)	62.8 (48.7–75.4)*	56.4 (30.8–65.3)
	L	60.6 (39.8–64.1)	56.1 (47.2–63.4)	55.9 (35.2–61.6)	49.2 (30.8–56.7)	59.8 (44.9–73.8)#	52.0 (40.7–66.2)

CA = carotids; BH = brain hemispheres; R = right; L = left. Data are expressed as median (1st quartile–3rd quartile).
* $p = 0.05$.
# $p < 0.029$.

in both groups. Quality of life survey results are displayed in the Supplementary Material.

Serum level analysis from subjects under leucine supplementation demonstrated that leucine levels increased from 34.2 nmol/L (23.1–45.5) to 63.0 nmol/L (30.2–120) at 12 months of treatment ($p = 0.049$). In contrast, isoleucine levels changed from 21.2 nmol/L (13.8–22.4) to 21.7 nmol/L (10.9–89.3) in this group (non-significant difference). At this same time, patients under isoleucine supplementation were evidencing a marked increase of both serum amino acids: levels of leucine increased from 27.9 nmol/L (19.9–37.0) to 40.1 nmol/L (27.2–58.9) ($p = 0.030$) while those of isoleucine increased from 14.4 nmol/L (11.3–20.7) to 88.5 nmol/L (20.8–207) ($p = 0.003$).

4. Discussion

The results of this trial suggest that hyperammonemia has a large impact on cerebral energy metabolism, in agreement with previous studies presented by Bessman and Bessman (1955); Cooper and Plum (1987); Lai and Cooper (1986); Ott and Vilstrup (2014). Furthermore, in the sample evaluated, isoleucine was able to reduce HE manifestations. The data demonstrate an increase of serum leucine levels at 12 months of supplementation in both groups, accompanied by gains in body assessment measures such as triceps skinfold after 8 and 12 months of supplementation.

Ammonia detoxification inside astrocytes causes an important influx of water to these glial cells due to GLN accumulation and the osmotic effect of this amino acid (Cichoz-Lach and Michalak, 2013). Concurrently, ammonia metabolism in muscle cells seems to be linked to strength loss, which is proportional to HE severity (Augusti et al., 2016; Merli et al., 2013). Thus, the results of strength restoration, triceps skinfold augmentation and HE grading improvement during isoleucine supplementation are inexplicable without considering a significant effect of BCAA on energy metabolism, thus decreasing cytotoxic brain edema and restoring muscle strength.

Taken together, these findings on muscular and astrocytic metabolism suggest that ammonia toxicity is related to a disruption in the cellular mechanisms involved in energy metabolism, especially in the brain. Glutamate dehydrogenase (GDH) activity depends on the amount of α-KG and energy from NAD/NADP provided by the TCA cycle, whereas glutamine synthetase activity requires ATP. However, excessive ammonia inhibits the α-ketoglutarate dehydrogenase complex (αKGDH) in this cycle (Ott and Vilstrup, 2014), shifting α-KG to GLN production and decreasing the α-KG oxidation to SuccinylCoA and CO_2, thus contributing to curbing aerobic energy production (Lai and Cooper, 1986) (Eq. (3)).

$$\alpha - KG^{2-} + CoASH^{4-} + NAD^{-} + H_2O \rightleftarrows \text{Succinyl}-CoA4^{-} + CO_3^{2-} + NADH^{2-} + H^{+} \quad (3)$$

Another piece of evidence that energy metabolism is the key to understanding how leucine and isoleucine can decrease HE manifestations is the formation of α-KG, which is produced when isocitrate is oxidized to α-KG and CO_2 (Eqs. (4) and (5)), a step that requires the bonding of a manganese ion to the carbonyl group before the decarboxylation. Thus, αKGDH inhibition by hyperammonemia leads not only to GLN accumulation but also to an interruption in the TCA cycle at α-KG formation (Fig. 1). Therefore, in advanced liver disease, both the high ammonia levels and the lack of BCAA may act together to impair α-KG production from oxalosuccinate (Eq. (5)). In tissues that depend on aerobic processes to obtain energy, such as brain cells, the reduced α-KG formation supposedly decreases the manganese uptake in this process. Consequently, the remaining manganese may accumulate in regions such as basal nuclei and cortical areas, a common finding in

Table 2
Clinical evaluation findings and laboratory tests comparing pretreatment values with the results at 8 and 12 months of supplementation.

Variables	Amino acid					
	Leucine group (n = 11)			Isoleucine group (n = 16)		
	Pretreatment	8 months	12 months	Pretreatment	8 months	12 months
MELD	12.0 (8.50–15.5)	11.0 (8.00–12.8)	12.0 (8.00–12.8)	11.0 (9.50–12.5)	11.5 (9.50–13.0)	12.0 (10.5–13.5)
Child-Pugh	8.00 (6.00–10.0)	7.00 (5.25–7.75)	7.00 (6.25–7.75)	7.00 (6.50–8.50)	6.00 (5.00–6.50)*	6.00 (5.00–7.50)
HE grade	1.00 (0.00–1.00)	0.00 (0.00–1.00)	0.00 (0.00–0.00)§	1.00 (0.00–1.00)	0.00 (0.00–0.00)§§	0.00 (0.00–0.00)§§§
Ammonia (μmol/L)	52.0 (27.8–78.2)	66.5 (44.0–94.0)	77.0 (46.5–114)	58.0 (26.5–95.5)	55.0 (13.8–94.5)	34.0 (20.8–66.0)

MELD = model for end-stage liver disease; Child-Pugh = Child-Pugh classification; HE = hepatic encephalopathy. Data are expressed as median (1st quartile–3rd quartile). The upper normal limit for plasma ammonia levels is 35 μmol/L.
* $p < 0.001$.
§ $p = 0.055$.
§§ $p = 0.008$.
§§§ $p = 0.004$.

Table 3
Body assessment measures comparing pretreatment values with the results at 8 and 12 months of supplementation.

Variables	Amino acid					
	Leucine group (n = 11)			Isoleucine group (n = 16)		
	Pretreatment	8 months	12 months	Pretreatment	8 months	12 months
Weight (kg)	82.2 (69.6–90.0)	92.4 (78.8–95.4)	91.6 (80.4–99.8)	69.7 (59.8–77.7)	78.0 (69.4–87.3)	75.6 (69.8–86.7)
BMI (kg/m^2)	30.1 (25.1–34.5)	31.5 (29.1–36.9)	31.6 (30.3–36.8)	26.0 (24.9–28.6)	29.7 (26.9–32.3)	29.4 (27.4–31.3)
AC (cm)	30.0 (26.8–34.5)	34.0 (30.2–36.5)	32.5 (29.5–35.8)	30.2 (26.6–32.2)	31.0 (28.4–33.9)	31.8 (27.0–32.9)
TSF (mm)	17.0 (10.0–19.8)	25.0 (22.0–32.5)*	27.0 (20.8–33.6)*	16.5 (8.50–21.0)	22.0 (17.0–30.0)*	23.5 (15.0–28.5)*
MAMC (cm)	25.7 (22.0–27.9)	24.2 (22.2–27.4)	23.9 (22.7–25.4)	25.5 (23.9–27.0)	23.7 (21.5–25.8)	23.9 (22.4–25.0)
CAMA (cm^2)	42.5 (30.4–53.5)	39.9 (29.2–49.6)	36.4 (31.2–41.9)	41.9 (35.3–48.3)	34.7 (26.8–43.2)	35.4 (29.8–40.6)
APMT (mm)	6.00 (4.50–9.00)	8.00 (6.50–9.00)	9.00 (7.00–10.0)	5.50 (4.75–7.25)	7.00 (5.00–9.00)	7.00 (6.00–8.00)
HGS (kg)	18.0 (16.5–22.0)	28.0 (22.0–38.8)§	28.0 (20.5–41.5)§§	25.5 (18.0–28.5)	33.0 (26.5–40.0)§§§	32.0 (26.5–41.0)§§

BMI = body mass index; AC = arm circumference; TSF = triceps skinfold; MAMC = mid-arm muscle circumference; CAMA = correct mid-arm muscle area; APMT = adductor pollicis muscle thickness; HGS = handgrip strength; kg = kilogram, kg/m^2 = kilogram per square meter, cm = centimeter, mm = millimeter. Data are expressed as median (1st quartile–3rd quartile).

* $p \leq 0.001$.
§ $p = 0.010$.
§§ $p = 0.002$.
§§§ $p = 0.001$.

patients with HE and acquired hepatocerebral degeneration.

$$\text{Isocitrate} + \text{NAD/NADP} \rightleftarrows \text{Oxalosuccinate} \quad (4)$$

$$\text{Oxalosuccinate} \overset{Mn^{+2}}{\rightleftarrows} \alpha - \text{KG} + CO_2 \quad (5)$$

As shown in Fig. 1, αKGDH inhibition is an additional cause of oxidative metabolism impairment in organs that depend on aerobic processes to obtain energy, such as the brain (Bak et al., 2013; Cooper and Plum, 1987; Lai and Cooper, 1986), thus impairing ATP generation from the TCA cycle. Despite the fact that GLN accumulation in astrocytes causes cytotoxic edema due to the osmotic effect of GLN (Brusilow et al., 2010; Parekh and Balart, 2015), it may be insufficient to account for the astrocytic swelling or the manganese deposits in HE, which only could be explained by additional disturbances, such as metabolic impairment and oxidative stress.

Even though brain perfusion control is not fully understood, there are important reports showing that cerebral blood flow (CBF) is regulated by astrocytes according to brain metabolism by controlling vasoconstriction and vasodilation (Belanger et al., 2011; Carmignoto and Gomez-Gonzalo, 2010). The GLU release by neurons raises intracellular Ca^{+2} in astrocytes, which activates the production of arachidonic acid and vasodilators such as prostaglandins and epoxyeicosatrienoic acids. On the other hand, arachidonic acid can be converted to 20-hydroxyeicosatetraenoic acid in arteriolar smooth muscle, resulting in vasoconstriction. Therefore, the direction toward vasodilation or vasoconstriction in the brain is mediated by metabolic conditions (Belanger et al., 2011; Carmignoto and Gomez-Gonzalo, 2010).

The findings obtained in this trial suggest that astrocytic dysfunction is probably the cause of the cerebral perfusion changes observed in HE. In the SPECT analysis, an augmentation of cerebral perfusion was documented after isoleucine supplementation at 8 and 12 months of treatment as shown in Fig. 3. The slope of the ascending curves obtained from brain hemispheres, depicting the CBF rise, constituted additional evidence of blood flow improvement at 8 months of isoleucine supplementation (Table 1). Cytotoxic edema recovery seems to constitute a reasonable explanation for the increased perfusion found at 8 and 12 months of treatment in the isoleucine group. However, the CBF improvement observed was greater at 8 months than at 12 months of treatment (Fig. 3, Table 1).

The imaging technique used in this work was the same employed by Iwasa et al. (2003), who also reported a CBF improvement in patients with cirrhosis under BCAA supplementation. Nevertheless, given that all BCAA (valine, leucine and isoleucine) were administered together in this previous study, the role of each one in this effect on cerebral perfusion was difficult to distinguish. In this present trial, long-term isoleucine supplementation has led to significant changes in cerebral perfusion, mainly in the white matter. Involvement of the cingulate gyrus and basal ganglia was observed as well. These brain areas were previously reported as being associated with HE symptoms, such as cognitive impairment, lack of attention, memory loss and deficits in visual-spatial perception (Catafau et al., 2000; Lockwood et al., 1993; O'Carroll et al., 1991).

The reason that the results from isoleucine supplementation were better than those obtained from leucine can only be understood by analyzing the effects of ammonia on the TCA cycle. Isoleucine and leucine can both be converted to Acetyl-CoA, but isoleucine is also converted to Succinyl-CoA, providing additional carbon to this cycle at the step when αKGDH is inhibited by ammonia (Bak et al., 2013; Holecek, 2010; Nissen et al., 2015; Ott et al., 2005). Due to this unique property of isoleucine, it can serve as an ideal substrate for maintaining TCA as a source of energy, even in the presence of hyperammonemia, by reestablishing oxaloacetate to form citrate from Acetyl-CoA (Johansen et al., 2007). Therefore, BCAA supplementation is capable of restoring the oxidative metabolism in neural cells, especially when a larger amount of isoleucine is administered, because it can reduce the disruption in ion transportation through the cell membranes, thus maintaining the osmotic regulation and probably reducing the cytotoxic edema observed in HE.

In addition to its role in TCA cycle maintenance, isoleucine is also involved in ammonia detoxification when α-KGDH is inhibited (Ott et al., 2005). This additional role of isoleucine might reduce the GLN concentration in astrocytes, thereby avoiding the cytotoxic edema. The evidence supporting this property of isoleucine is that the subjects who received isoleucine presented elevated serum levels of both isoleucine and leucine. On the other hand, leucine supplementation led to an increase only in this same amino-acid serum level, probably due to the fact that leucine supplies Acetyl-CoA only for the TCA cycle, which requires isoleucine to provide Succinyl-CoA and keep the cycle running (Fig. 1) (Johansen et al., 2007; Ott et al., 2005).

Both leucine and isoleucine supplementations improved the quality of life related to physical functioning, most likely by restoring energy generation and strength, as shown by the handgrip strength elevation in both groups, indicating that some symptoms were ameliorated. Although the increase in cerebral perfusion was more pronounced at 8 months, the clinical improvement was maintained until the end of the trial and the subjects did not suffer HE deterioration. Therefore, the

increase obtained in cerebral perfusion may be a compensatory mechanism induced by isoleucine in the first months, leading to a partial restoration in the metabolic coupling between cerebral regions. The results suggest that this mechanism was activated to a lesser degree when the subjects achieved improvement in their HE manifestations, a hypothesis in line with relevant studies on this issue (Catafau et al., 2000; Zafiris et al., 2004). Last but not least, four subjects in the leucine group exited the trial because they had experienced nausea and vomiting. The lack of such terminations in the isoleucine group leads to the suspicion that isoleucine preparations may be more palatable than leucine preparations.

In conclusion, the results of this double-blind randomized clinical trial showed that isoleucine supplementation facilitates the achievement of a better impact on brain perfusion and HE grade than that obtained by leucine, suggesting that patients with cirrhosis and HE should receive supplements enriched with a higher level of isoleucine than leucine.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2018.03.028.

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MATERIAL COMPLEMENTAR

Supplementary Material

Randomization plan

The subjects were randomized on a 1:1 basis. The randomization plan was obtained from http://www.randomization.com and a list for 50 patients receiving two different treatments was created (Dallal, 2013). The amino acid packets were only marked with the labels “A” or “B” and only the person responsible for the manufacturing process knew which amino acid the subjects were receiving, assuring that all the assessments were blinded during the trial. After defining the group to which each patient would belong, the supplement “A” or “B” was given in packets of 10 g.

Subject’s flow chart

Sixty-seven outpatients were invited to participate. Seventeen subjects missed the follow-up before starting the trial, so the supplementation began with 50 individuals (25 in each group). Four patients from each group did not return for the study appointments. Eight subjects were excluded from the leucine group (4 for nausea and vomiting, 1 for feeling HE worsening, 1 for hepatocellular carcinoma, 1 for diarrhea, 1 for death caused by gastrointestinal bleeding). Two subjects were excluded from the isoleucine group (1 for liver transplantation and 1 for death after pulmonary infection and sepsis). Thus, 13 subjects in the leucine group and 19 subjects in the isoleucine group finished the 12 months of supplementation. However, five subjects were not included in the study analysis for not completing the study exams (2 from the leucine group and 3 from the isoleucine group). The subjects’ characteristics are summarized in Table 1 and their flow diagram throughout the study is shown in Figure 2.

Electroencephalogram

Electroencephalogram was done just before the treatment in order to diagnose Grade 0 hepatic encephalopathy. Before the trial, interictal electroencephalogram was performed with a 32-channel recorder (Nihon Kohden, Tokyo, JPN). Electrodes were positioned as stated by the 10-20 international system of electrode placement. The exam was performed within 20 minutes, while hyperventilation and photic stimulation were both applied. The results were analyzed by a specialist who was not aware of the subjects' data. Electroencephalographic signs of HE in the background activity were used to categorize the results as normal or abnormal (Amodio et al., 1999).

Single Photon Emission Computed Tomography (SPECT) analysis

The data were examined using SPM12 (Statistical Parametrical Mapping 12, Wellcome Department of Cognitive Neurology, University College, London, UK) under Matlab 8.5 (Mathworks Inc., Sherborn, MA, USA). Each pixel was thresholded at 10% of the mean pixel intensity across all slices. SPECT scans were standardized by a 12-parameter affine transformation in order to apply them on a template and to reformate the data to 8-bit images of 128 x 128 x 128 voxels, each with a voxel size of 3 x 3 x 3 mm3. Spatially normalized images were then smoothed by a Gaussian filter (FWHM 3.0 mm). SPECT was performed at 1, 8 and 12 months of treatment.

Dynamic brain scintigraphy

A dynamic set of 40 frames (3 seconds per frame) was acquired in a 64 x 64 matrix in anterior view. Digital images were analyzed in MatLab 8.5. Dynamic images were visually assessed and only the first 20 images obtained from the 99mTc achieving the carotids were selected to analysis (1 minute of cerebral blood flow). Regions of interest from hemispheres and carotids were manually defined and applied by the operator. The size of regions was defined to include an entire hemisphere and each region of interest was mirrored and applied

to the contralateral one. Regions of interest of carotids constituted by 2 x 2 pixels were defined to avoid inclusion the thyroid and salivary glands within the region. Average count activity *versus* time curves from those regions were plotted and then utilized to assess the initial slope of the curve, corresponding to the 99mTc arrival (Matsuda et al., 1992; Zaknun et al., 2008). It was also performed at 1, 8 and 12 months of treatment, in the same week that the SPECT exam was done.

Nutritional assessment

Nutritional status was evaluated through anthropometric measures and handgrip strength. The anthropometric measurements collected were weight, height, arm circumference, triceps skinfold and adductor pollicis muscle thickness. Body mass index was calculated from the weight divided by the square of the height. The mid-arm muscle circumference and the correct mid-arm muscle area were obtained from arm circumference and triceps skinfold (Blackburn and Thornton, 1979; Callaway et al., 1988; Harrison et al., 1988). Handgrip strength from non-dominant hand was registered by a dynamometer (Saehan grip - Saehan Corporation, SK). Three attempts were required, and the highest value was registered. The nutritional assessment was done bimonthly, with the clinical evaluations.

Amino acid serum levels

The aqueous mobile was Amino Acid Mobile Phase Kit, Na Phase (Shimadzu, JPN) consisting of phase A: buffer trisodium citrate dehydrate (19.6g), ethanol (55.3g), perchloric acid (60%), octanoic acid (0.1 ml) and water (0.91 Kg), phase B: buffer trisodium citrate dehydrate (19.6g), boric acid (12.4g), sodium hydroxide (5.2g), octanoic acid (0.1 ml) and water (0.97 Kg), phase C: sodium hydroxide (4g) and water (0.50 Kg). The injection volume was 20 µl. Flow rate was set at 0.6 ml/min (phase A) and 0.2 ml/min (phase B and C). The sample was diluted in NaS buffer pH 2.2 and filtered by GV Millex (Millipore™, USA) before

infusion in the equipment. Shimadzu LC-10A/C-47A system was applied to data analysis using the ortho phthalaldehyde derivatization method.

Quality of life survey

Quality of life was assessed using the Medical Outcomes Study 36-item Short-Form General Health Survey validated for Portuguese (Ciconelli et al., 1999). The questionnaire consists of 11 questions and 36 items comprising 8 scales subdivided into two categories: physical component summary and mental component summary. The physical component summary is composed of physical functioning, role-limitation physical, bodily pain and general health. The mental component summary is composed of vitality, social functioning, emotional-role limitation and mental health. Short Form Health Survey (SF-36) scales range from 0 (lowest) to 100 (highest), with higher scores indicating better health-related quality of life. This survey was applied before the supplementation and at 12 months of treatment (Table 4).

Table 4 - Health-related Quality of Life scores measured before and at the end of the trial

	Amino acid			
	Leucine group (n= 11)		Isoleucine group (n= 15)	
Scales	Pretreatment	12 months	Pretreatment	12 months
Physical functioning	40.0 (21.2 - 83.8)	90.0 (76.2 - 95.0) **	80.0 (50.0 - 97.5)	95.0 (90.0 - 100) *
Physical role limitation	0.00 (0.00 - 50.0)	25.0 (0.00 - 100)	100 (50.0 - 100)	100 (50.0 - 100)
Bodily pain	100 (36.0 - 100)	84.0 (57.5 - 100)	84.0 (34.0 - 100)	100 (87.0 - 100)
General health	45.0 (26.0 - 77.0)	80.0 (39.5 - 88.5)	77.0 (51.0 - 91.0)	82.0 (68.5 - 92.0)
Physical component summary	165 (114 - 272)	261 (174 - 380)	301 (234 - 376)	370 (318 - 377)
Vitality	55.0 (20.0 - 85.0)	80.0 (37.5 - 95.0)	85.0 (55.0 - 92.5)	90.0 (82.5 - 97.5)
Social functioning	50.0 (25.0 - 100)	100 (68.8 - 100)	100 (73.8 - 100)	100 (87.5 - 100)
Emotional role limitation	100 (18.8 - 100)	66.7 (0.00 - 100)	100 (100 - 100)	100 (78 - 100)
Mental health	60.0 (36.0 - 78.0)	80.0 (56.0 - 87.0)	80.0 (60.0 - 94.0)	88.0 (80.0 - 94.0)
Mental Component Summary	275 (96.8 - 366)	318 (170 - 384)	358 (299 – 378)	374 (304 – 391)

Data are expressed as median (1st quartile – 3rd quartile). The isoleucine group analysis was performed with 15 individuals.* p=0.030; **p= 0.008.

ANEXOS

ANEXO 1: Aprovação do Comitê de Ética em Pesquisa (2012)

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Registrado no Ministério da Saúde
em 30 de abril de 1997

Botucatu, 03 de setembro de 2012 Of. 432/2012

Ilustríssimo Senhor
Prof. Dr. Carlos Antonio Caramori
Departamento de Clínica Médica da
Faculdade de Medicina de Botucatu

Prezado Prof. Caramori,

De ordem do Senhor Coordenador, informo que o Projeto de Pesquisa, **(Protocolo CEP 4334-2012)** "Efeito da suplementação com aminoácidos de cadeia ramificada na composição corporal de portadores de cirrose com encefalopatia hepática", a ser conduzido por Lais Augusti, orientada por Vossa Senhoria, Co-orientada pelo Prof. Dr. Fernando Gomes Romeiro, recebeu do relator parecer favorável, aprovado em reunião de 03/09/2012.

Situação do Projeto: APROVADO. Os pesquisadores deverão apresentar ao CEP ao final da execução do Projeto o "Relatório Final de Atividades".

Atenciosamente,

Alberto Santos Capelluppi
Secretário do CEP

ANEXO 2: Aprovação do Comitê de Ética em Pesquisa (2014)

unesp
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Registrado no Ministério da Saúde em 30 de abril de 1997

Botucatu, 17 de julho de 2014 Of. 94/2014-CEP

Ilustríssimo Senhor
Prof. Dr. Fernando Gomes Romeiro
Departamento de Clínica Médica
Faculdade de Medicina de Botucatu

Com referência Projeto de Pesquisa **(Protocolo CEP 4334-2012)** "Efeito da suplementação com aminoácidos de cadeia ramificada na composição corporal de portadores de cirrose com encefalopatia hepática", conduzido por Laís Augusti e aprovado por esse CEP em 03/09/2012, sofreu algumas alterações abaixo relacionadas, e aprovado pela Coordenação deste CEP nesta data.

1. Mudança de Título do Projeto passando a denominar-se: (Protocolo CEP 4334-2012) Ensaio Clínico prospectivo, randomizado, duplo cego e controlado sobre os efeitos da Leucina *versus* Isoleucina no tratamento da encefalopatia hepática de acordo com o estado nutricional de portadores de cirrose"
2. Novo orientador: Prof. Dr. Fernando Gomes Romeiro
3. Novo Co-orientador: Prof. Dr. Carlos Antonio Caramori
4. Mudança proposta e aprovada: "Leucina *versus* Isoleucina"
5. Aprovada a nova versão do: Termo de Consentimento Livre e Esclarecido.

O CEP solicita aos pesquisadores o envio do Relatório Final de Atividades, tão logo o estudo esteja concluído.

Atenciosamente,

Profª Drª Silvana Andréa Molina Lima
Coordenadora do CEP

ANEXO 3: Aprovação do Comitê de Ética em Pesquisa (2015)

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Ética

Registrado no Ministério da Saúde
em 30 de abril de 1997

Botucatu, 06 de Agosto de 2015 OF. 98/2015-CEP

Ilustríssimo Senhor
Prof. Dr. Fernando Gomes Romeiro
Departamento de Clínica Médica
Faculdade de Medicina de Botucatu.

Caro Prof. Fernando,

Com referência ao Projeto de Pesquisa: **Protocolo SIPE 4334/2012 "Ensaio Clínico prospectivo, randomizado, duplo cego e controlado sobre os efeitos da Leucina versus Isoleucina no tratamento da encefalopatia hepática de acordo com o estado nutricional de portadores de cirrose"** aprovado por este colegiado em 03/09/2012, esclareço que este, foi dividido em 2 partes à saber:

Parte I: **Protocolo SIPE 4334/2012-A = Sub-Projeto I:** "Avaliação do estado nutricional, ingestão protéica e qualidade de vida de indivíduos cirróticos com encefalopatia hepática " o qual foi desenvolvido com dados da análise baseline dos indivíduos incluídos no ensaio clínico e coletados dados sobre a composição corporal, ingestão alimentar, qualidade de vida e exames laboratoriais pré suplementação e que resultou na Dissertação de Mestrado de **Laís Augusti** sobre orientação do Prof. Dr. Fernando Gomes Romeiro, já entregue ao CEP o respectivo **"Relatório Final de Atividades"**, cuja aprovação se deu em 08/07/2015.

Parte II: **Protocolo SIPE 4334/2012-B = Sub-Projeto II:** "Suplementação de diferentes aminoácidos de cadeia ramificada no tratamento da encefalopatia hepática em portadores de cirrose: Ensaio clínico,randomizado, duplo cego e controlado" cujos dados são os resultados de 01 ano de suplementação na composição corporal, qualidade de vida e exames laboratoriais dos participantes do estudo, e que está sendo realizado para Defesa de Tese de Doutorado de **Laís Augusti** sobre orientação do Prof. Dr. Fernando Gomes Romeiro.

Atenciosamente,

Profª Drª Silvana Andréa Molina Lima
Coordenadora do CEP