Oral acetyl-L-carnitine therapy reduces fatigue in overt hepatic encephalopathy: a randomized, double-blind, placebo-controlled study[1–3]

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ABSTRACT

Background: Fatigue is frequently reported in hepatic encephalopathy (HE) and may be related to hyperammonemia. Acetyl-L-carnitine (ALC) offers neuroprotective benefits and improves mitochondrial energetics and function.

Objective: This study evaluated the effect of exogenous ALC on physical and mental fatigue, fatigue severity, and physical activity in patients with mild and moderate hepatoencephalopathy (HE1 and HE2, respectively).

Design: A total of 121 patients with overt HE were recruited to the study and were subdivided into 2 groups according to their initial HE grade [HE1 (n = 61) or HE2 (n = 60)]. Thirty-one patients with HE1 and 30 with HE2 received 2 g ALC, and 30 patients with HE1 and 30 patients with HE2 received placebo twice a day for 90 d. All patients underwent clinical and laboratory assessments and automated electroencephalogram analysis.

Results: At the end of the study period, the ALC-treated patients in the HE1 group showed significantly better improvement than did the placebo group in mental fatigue score (−1.7 compared with −0.3; $P < 0.05$), the fatigue severity scale (−6.4 compared with 2.3; $P < 0.001$), 7-d Physical Activity Recall questionnaire score (17.1 compared with −2.5; $P < 0.001$), and Short Physical Performance Battery (2.1 compared with 0.2; $P < 0.001$); the HE2 group showed significantly better improvement in the fatigue severity scale (−8.1 compared with −5.1; $P < 0.001$) and 6-min walk test (19.9 compared with 2.3; $P < 0.05$). Significant decreases in NH_4^+ were observed in both groups ($P < 0.001$).

Conclusion: Patients with HE treated with ALC showed a decrease in the severity of both mental and physical fatigue and an increase in physical activity. This trial was registered at clinicaltrials.gov as NCT01223742. *Am J Clin Nutr* 2011;93:799–808.

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric complication of cirrhosis. Overt HE can be diagnosed clinically, and a mild-to-moderate grade of disease might be present in a considerable proportion of ambulatory patients with cirrhosis. Overt HE is a syndrome of neurologic and neuropsychiatric abnormalities. Affected patients exhibit alterations in psychomotor, intellectual, cognitive, emotional, behavioral, and fine motor function. Fatigue is frequently reported in HE and can also be related to hyperammonemia (1). Ammonia is recognized as a crucial component in the pathogenesis of HE, but other factors, such as oxygen free radicals, circulating opioid peptides, nitric oxide, inflammatory cytokines, reduction in serotoninergic neurotransmitters, depletion of endogenous antioxidants, neurosteroids, and manganese, are also implicated in the development of the disease (2). In recent years, fatigue has been researched as the main symptom of elevated ammonia in HE (1, 3). The treatments that remove ammonia from the body or that decrease ammonia production and absorption through the gastrointestinal tract improve mental status and cognitive function, but no effects have yet been shown in fatigue treatment. Our previous study showed a protective effect of L-carnitine against ammonia-evoked encephalopathy in cirrhotic patients, and another study showed that acetyl-L-carnitine (ALC) administration improved neurologic symptoms and plasma variables in selected cirrhotic patients with hepatic coma. Finally, other studies showed that ALC treatment reduces fatigue in the elderly and in centenarians (4–7). ALC is an endogenous molecule synthesized in mitochondria by the enzyme ALC transferase and is the predominant acylcarnitine in normal tissues. Acylcarnitine is the fatty acid–bound form of L-carnitine, which has an important role in the transport of long-chain fatty acids into mitochondria and in their β-oxidation (8–10). Serum acylcarnitine is mainly composed of short-chain fatty acid L-carnitine, especially ALC. Although 99% of the amount of L-carnitine is intracellular, the relation between serum acylcarnitine and free L-carnitine is highly sensitive to intramitochondrial metabolic alterations (11). ALC treatment restores the altered neurochemical abnormalities, cerebral energy metabolites in ischemia and aging and, in particular, ammonia-induced cerebral energy depletion (12). It also facilitates the removal from the mitochondria of excess short- and medium-chain fatty acids that accumulate during

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metabolism (13). Some of ALC's proposed neuroprotective benefits involve improved mitochondrial energetics and function, antioxidant activity, stabilization of membranes, protein and gene expression modulation, and enhancement of cholinergic neurotransmission (14). Patients with fatigue show reduced exercise tolerance and postexercise fatigue induced by minimal physical activity, which suggests decreased muscle function. During physical activity, the rate of free radical formation may overcome the various protective defense mechanisms and induce systemic oxidative stress through plasma accumulation of secondary products of lipid peroxidation (15). The aim of this study was to evaluate the effect of exogenous ALC on physical and mental fatigue, fatigue severity, and physical activity in patients with mild and moderate encephalopathy.

SUBJECTS AND METHODS

Subjects

A total of 121 cirrhotic patients (22 with hepatitis B virus infection, 65 with hepatitis C virus infection, 9 with alcoholism, and 25 with cryptogenetic cirrhosis) meeting the following inclusion criteria were enrolled in the study:

1) Chronic hepatitis with spontaneous manifest HE (mental state grade 1 or 2 according to the West Haven criteria) and a Number Collection Test-A performance time >30 s
2) Hyperammonemia (venous ammonia concentration >50 mmol/L)
3) Cooperative, hospitalized adult patients with liver cirrhosis diagnosed by clinical, histologic, and ultrasonographic findings (reduced dimensions of the liver as well as splenomegaly) and esophageal varices at stages 2 and 3 observed by endoscopy

Exclusion criteria

The exclusion criteria were as follows:

1) Major complications of portal hypertension, such as gastrointestinal blood loss, hepatorenal syndrome, or bacterial peritonitis
2) Acute superimposed liver injury
3) Patient with other neurologic disease and metabolic disorders, diabetes mellitus, unbalanced heart failure, and/or respiratory failure or end-stage renal disease
4) Alcoholic-toxic cirrhosis because toxic brain damage may interfere with the assessment of HE
5) Severe HE
6) Administration of anti-HE medications, such as neomycin and branched-chain amino acids
7) Any additional precipitating factors, such as high protein intake (additional high-protein meals), constipation, or intake of psychostimulants, sedatives, antidepressants, benzodiazepines, benzodiazepines-antagonists (flumazenil), neuromuscular blocking agents, and certain antibiotics
8) Patients with fever, sepsis, or shock were also excluded to avoid variations caused by body temperature
9) Illiteracy

The study protocol was received and approved by the Institutional Review Board of the Hospital following the guidelines of the 1975 Declaration of Helsinki (16). All patients gave written informed consent before any study procedures were initiated.

Study design

This was a randomized, double-blind, placebo-controlled study. The study was performed between June 2002 and December 2007. Patients meeting the inclusion criteria were randomly assigned to either a 90-d treatment with ALC (group A) or placebo (group B). Randomization was based on a computer-generated list. All study subjects were subdivided into 2 groups on the basis of the initial grade of HE: mild (grade 1; HE1) or moderate (grade 2; HE2) according to the West-Haven criteria (17). Group A consisted of patients with initial HE1 (ALC group: n = 31; placebo group: n = 30 placebo); group B consisted of patients with initial HE2 (ALC group: n =30 patients; placebo group: n =30 patients). The effectiveness of therapy was compared and evaluated separately in the different subgroups.

Methods

Clinical and laboratory assessment and automated electroencephalogram (EEG) analysis were performed for all patients. The diagnosis of HE grade was based on the evaluation of consciousness, intellectual functions, behavior, and neuromuscular functions and was made when appropriate laboratory and diagnostic testing excluded other causes of mental status changes. The investigators were blinded to the patients' ammonia concentrations. Patients whose clinical course was not consistent with HE were excluded. Mental status was assessed and graded on admission according to the West Haven criteria introduced by Conn (18).

Prerandomization phase

The subjects were required to document all caloric intake with the use of a diary, which was completed every 2 d. This prerandomization period was designed to nullify the effects of dietary changes on metabolic markers. During the initial 2-wk phase, subjects were instructed by a dietitian to follow an ad libitum diet as follows: 25–30% total fat, <7% saturated fat, ≤10% polyunsaturated fat, ≤20% monounsaturated fat, 50–60% of total energy as carbohydrate, ≈15% of total energy as protein, and <200 mg cholesterol/d (19). Patients were seen by a dietitian every month; at each visit the dietitian provided instructions on dietary intake recording procedures as part of a behavior-modification program, and the patients' resulting food diaries were later used for counseling. All patients in both groups were given the same 1600-calorie diet and prescribed exercise plan. The subjects underwent weekly visits throughout the treatment period to assess adherence to the study protocol, to measure blood pressure, and to record adverse events.

Randomization phase

Throughout the trial, ALC was supplied in vials with 2 g ALC taken orally twice a day. All drugs and placebos were identical in appearance, and neither the investigators nor the patients were informed of the selected agent until the end of the study phase.

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Dosing instructions were provided with each patient pack. All trial medication was instructed to be taken as prescribed. Subjects were considered compliant if the number of returned vials was between 80% and 120% of the planned treatment regimen. For the duration of the trial, any concomitant drugs were administered at the lowest possible therapeutic dose and, as much as possible, were not changed. Concomitant medications throughout the study included diuretics and β-blockers (**Table 1**).

Fatigue assessment

Severity of fatigue

Severity of fatigue was measured by the Fatigue Severity Scale (FSS). The FSS is a self-assessed 9-question scale ranging from 1 (no signs of fatigue) to 7 (most disabling fatigue). Here, the total score ranged from 9 to 63 and is directly related to the severity observed (20).

Nature of fatigue

Wessely's test and Powell's test were used to examine fatigue, both mental and physical. The Wessely and Powell score consists of 2 scales measuring physical fatigue [8 items scored from 0 (no fatigue) to 2 (highest possible fatigue); total score range: 0–16] and mental fatigue (5 items; total score range: 0–10) (21).

Measures of physical activity

Physical activity was assessed by using the 7-d Physical Activity Recall questionnaire (7-d PAR) and a pedometer. On the 7-d PAR, the patients self-reported moderate, hard, and very hard periods of physical activity performed during the 7-d period. The total duration of physical activity classified as "at least moderate intensity" was computed and used for analysis. This self-administered questionnaire has been shown to provide valid and reliable estimates of habitual physical activity (22). A pedometer (Digiwalker SW-200; Yamax Corporation, Tokyo, Japan) was used to obtain an objective measure of ambulatory physical activity. The subjects were instructed to wear the pedometer daily for 1 wk before treatment and for 1 wk before their scheduled 3-mo follow-up assessment. They were provided a diary to record their daily steps. The data are presented as the average steps taken daily. Physical function was assessed by using both performance-based and self-reported measures. The 6-min walk test (6MWT) measures the distance walked in 6 min on level ground, with stops to rest as needed. The subjects were told that the purpose of this test was to determine the distance they could walk in 6 min. They were instructed to "walk at their own pace in order to cover as much ground as possible" (23). The Short Physical Performance Battery (SPPB) is a battery of tests that has been used to assess lower extremity function in the older population (24). This battery uses a scale from 0 (poor) to 12 (excellent) to summarize the performance of 3 tasks (a 4-m walk, standing balance, and rising from a chair). For the 4-m walk, the subject walks a distance of 4 m at their normal pace to determine gait speed, computed as the time to complete the 4-m walk. For the standing balance test, the subjects placed their feet in a side-by-side position, followed by a semitandem position (heel of one foot along the side of the big toe of the opposite foot) and a tandem position (heel of one foot directly in front of the other). The subjects were required to hold the side-by-side position for 10 s before advancing to the semitandem position and to hold the semitandem position for 10 s before advancing to the tandem position. For the chair rise test, the subject was seated in a chair that was 18 in (≈45.72 cm) tall, with their arms crossed, and how quickly they could stand 5 times from sitting in the chair was assessed (24).

TABLE 1
Concurrent medications taken by the patients at enrollment[1]

	Group A: ALC (n = 61)	Group B: placebo (n = 60)
	n	*n*
β-Blockers	20	18
Insulin	4	4
Furosemide	18	16
Lactulose	10	13

[1] ALC, acetyl-L-carnitine. There were no significant differences between the 2 treatment groups.

Neurophysiologic assessment

The EEG was recorded by using standardized techniques. Five electrodes were attached to the skin at the positions T3, T4, O1, O2, and Cz according to the international "10-20 system." Electrode impedance was kept lower than 5KQ. After the usual handpass filters (0.53–35 Hz) were applied, 2 runs of 100 s each were recorded and compared for reproducibility. Patients were graded into different studies of HE according to their mean dominant frequency (MDF) and the relative powers of delta and theta activity (25). The EEG is the only test that classifies HE in 5 grades of severity (from normal to coma), just as the clinical grading: grade 0 (normal, regular alpha rhythm), grade 1 (irregular background activity, alpha and theta rhythm), grade 2 (continuous theta activity, occasionally delta activity), grade 3 (prevalence of theta activity, transient polyphasis complexes of spikes and slow waves), and grade 4 (continuous delta activity, abundant complexes of spikes and slow waves) (26).

Liver function assessment

The Child-Pugh score was determined to assess the severity of cirrhosis, including 3 biochemical variables (serum albumin, bilirubin, and prothrombin time) and 2 clinical characteristics (presence or absence of ascites and clinical HE). A patient had Child-Pugh score A cirrhosis if the score was $\leq$6 points, Child-Pugh B cirrhosis if the score was 7–9 points, and Child-Pugh C cirrhosis if the score was $>$9 points. Patients without signs of ascites were scored as 2 points for ascites (27). We also evaluated the presence and severity of the porto-systemic shunt by portal vein flow, presence and size of the esophageal varices, and splenic size.

Venous ammonia concentration

Ammonia was measured by enzymatic determination of glutamate dehydrogenase in a rapid and interference-free photometric determination (340 nm) of NH_4^+ in native blood plasma according to the Da Fonseca-Wollheim method (28). For reasons of safety, blood was immediately refrigerated and transported to the laboratory for immediate measurement of NH_4^+ (within 15 min of blood withdrawal).

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Efficacy assessment

Throughout the randomization phase of the study, thrice weekly alimentary diary cards were used to collect efficacy data. The primary efficacy measures were changes in activity, motivation, and physical and mental fatigue severity. Measurements were made at the beginning and at the end of the study period. Data were collected in the morning, after an overnight fast. Activity, motivation, physical and mental fatigue, and the severity of fatigue were assessed before and after treatment.

Tolerability assessment

Laboratory assessments were monitored on days 0, 30, 60, and 90. These data included blood tests (hemoglobin, hematocrit, white blood cell count, and thrombocytes) and liver function tests [alanine aminotransferase (AST), aspartate aminotransferase (AST), γ-glutamyl-transpeptidase, cholinesterase activity, serum bilirubin concentrations, prothrombin time, and partial thromboplastin time]. Electrocardiogram and blood pressure were monitored with the use of standard techniques.

Statistical analysis

We calculated that a simple size of ≥25 patients in each arm would be required to detect a difference in improvement in HE, that is the proportion of patients with HE at 2 mo, with a 5% type 1 error and 90% power for a 2-tailed log-rank test. Descriptive statistics were prepared from the study sample, and the results are expressed as means ± SDs. The statistical significance in contingency tables was evaluated by using chi-square and Fisher exact test. Student's *t* test was used for unpaired data, and one-factor analysis of variance and the Mann-Whitney rank-sum test were used for comparisons of continuous variables. The statistical analyses were performed by using appropriate tests for repeated measures and by controlling for multiple comparisons by correction with the Duncan procedure. Differences in tolerability were assessed with a chi-square test comparing the proportions permanently withdrawn from all study drugs or placebos. Statistical Analysis System software version 6.11 (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Baseline values

The 2 groups were homogeneous for demographic characteristics, etiology, casting of disease, Child-Pugh grade, anamnestic, and diagnostic criteria (**Table 2**). Differences in the composition of the 2 groups with respect to precipitant factors might be minimized, because the patient population was well defined by inclusion and exclusion criteria. Serum NH_4^+ fasting concentrations were not significantly different before the treatment. No statistically significant differences were observed between the 2 groups about prothrombin time, serum albumin, bilirubin, AST, and ALT. No statistically significant differences in the administered neuropsychologic test or in the EEG were observed between the 2 groups.

Neurophysiologic response

In the comparison between group A (treated with ALC) and group B (treated with placebo) we observed in HE1 an improvement in EEG grading in 45% of patients in the ALC group and in 13% of patients in the placebo group [odds ratio (OR): 5.35; 95% CI: 1.50, 19], whereas in HE2 there was an improvement in EEG grading in 66% of patients in the ALC group compared with 27% of patients in the placebo group (OR: 5.5; 95% CI: 1.81, 1.37) (**Table 3**).

TABLE 2
Baseline characteristics of the patients[1]

Characteristic	Group A: ALC (n = 61)	Group B: placebo (n = 60)
Sex (male/female)	32/29	33/27
Age (y)	40–66	41–67
SBP (mm Hg)	140 ± 16[2]	136 ± 18
DBP (mm Hg)	80 ± 7	77 ± 9
HR (beats/min)	87 ± 16	84 ± 15
NCT-A (s)	48 ± 12	47 ± 14
Cirrhosis etiology (n)		
Posthepatitis B	12	10
Posthepatitis C	30	35
Alcoholism	5	4
Cryptogenetic	14	11
Child-Pugh class		
A	20	21
B	36	28
C	5	11

[1] ALC, acetyl-L-carnitine; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; NCT-A, number collection test-A. There were no significant differences between the 2 treatment groups.
[2] Mean± SD (all such values).

L-Carnitine in plasma and urine

In the ALC group, significant differences were observed in the following markers after treatment compared with baseline in both HE1 and HE2: free plasma L-carnitine ($P < 0.001$), plasma concentrations of total plasma L-carnitine ($P < 0.001$), plasma long-chain acylcarnitine (LCAC) ($P < 0.001$), and short-chain acylcarnitine (SCAC) ($P < 0.05$). Only in HE2 did we observe significant differences in free urinary L-carnitine ($P < 0.001$). In the placebo group (in both HE1 and HE2), the plasma concentrations of free L-carnitine and LCAC and the urinary excretion of free L-carnitine and SCAC were not significantly different from baseline. At the end of the study period, compared with placebo, the ALC-treated patients showed significant improvements in the following markers in HE1 and HE2: free plasma L-carnitine (3.8 compared with 0.7 μmol/L in HE1 and 5.4 compared with 0.8 μmol/L in HE2; $P < 0.001$), plasma concentrations of total L-carnitine (4.4 compared with 0.9 μmol/L in HE1 and 6.2 compared with 1.1 μmol/L in HE2; $P < 0.001$), and plasma SCAC (0.4 compared with 0.1 μmol/L in HE1 and 0.5 compared with 0.2 μmol/L in HE2; $P < 0.001$) (**Table 4**).

Effects of ALC on fatigue

At the end of treatment in the group treated with ALC in HE1, we observed significant differences from baseline in the physical fatigue score ($P < 0.001$), mental fatigue score ($P < 0.001$), and fatigue severity scale ($P < 0.001$); in HE2, we observed significant differences in the physical fatigue score ($P < 0.05$),

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TABLE 3
Electroencephalogram, fatigue, and physical results in the patient subgroups[1]

Medication	Improved	Not improved	Total subjects	Improved:not improved	Odds ratio
	n (%)	*n*	*n*		
Electroencephalogram results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	20 (66)	10	30	2	5.5
Group B: placebo	8 (27)	22	30	0.36	
Total	28	32	60		
Initial HE grade 1					
Group A: ALC	14 (45)	17	31	0.82	5.35
Group B: placebo	4 (13)	26	30	0.15	
Total	18	43	61		
Fatigue results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	25 (83)	5	30	5	10
Group B: placebo	10 (33)	20	30	0.5	
Total	35	25	60		
Initial HE grade 1					
Group A: ALC	20 (64)	11	31	1.81	7.27
Group B: placebo	6 (20)	24	30	0.25	
Total	26	35	61		
Physical activity results in the patient subgroups					
Initial HE grade 2					
Group A: ALC	23 (77)	7	30	3.28	7.66
Group B: placebo	9 (30)	21	30	0.42	
Total	32	28	60		
Initial HE grade 1					
Group A: ALC	22 (71)	9	31	2.44	9.77
Group B: placebo	6 (20)	24	30	0.25	
Total	28	33	61		

[1] ALC, acetyl-L-carnitine; HE, hepatic encephalopathy.

mental fatigue score ($P < 0.001$), and fatigue severity scale ($P < 0.001$). After 90 d, significant differences were observed between the ALC-treated patients in HE1 and the placebo-treated patients in mental fatigue score (−1.7 compared with −0.3; $P < 0.05$) and fatigue severity scale (−6.4 compared with 2.3; $P < 0.001$), whereas in HE2 significant differences were observed in the fatigue severity scale (−8.1 compared with −5.1; $P < 0.001$) (**Table 5**). In the comparison between group A (treated with ALC) and group B (treated with placebo), we observed in HE1 an improvement in fatigue severity in 64% of patients in the ALC group compared with 20% of patients in the placebo group (OR: 7.27; 95% CI: 2.44, 23.16), whereas in HE2 there was an improvement in fatigue severity in 83% of patients in the ALC group compared with 33% of patients in the placebo group (OR: 10; 95% CI: 2.94, 34) (Table 3).

Effects of ALC on physical activity

At the end of treatment in the group treated with ALC in HE1 we observed significant differences in 7D/PAR ($P < 0.05$) and SPPB ($P < 0.001$); in HE2 the significant differences were in 7D/PAR ($P < 0.001$), pedometer ($P < 0.001$), and SPPB ($P < 0.001$). After 90 d, significant differences between the ALC-treated patients in HE1 and the placebo-treated patients were observed in 7D/PAR (17.1 compared with −2.5; $P < 0.001$) and SPPB (2.1 compared with 0.2; $P < 0.001$); in HE2 the significant differences were in 6MWT (19.9 compared with 2.3; $P < 0.05$) (Table 5). In the comparison between group A (treated with ALC) and group B (treated with placebo), we observed in HE1 an improvement in physical activity in 71% of patients in the ALC group compared with 20% of patients in placebo group (OR: 9.77; 95% CI: 2.99, 31.94), whereas in HE2 there was an improvement in physical activity in 77% of patients in the ALC group compared with 30% of patients in the placebo group (OR: 7.66; 95% CI: 2.42, 24.24) (Table 3).

Biochemical response

Effects of ALC on ammonia

In HE1 and HE2 at the end of treatment with ALC, we observed a significant decrease in NH_4^+ ($P < 0.001$). Moreover, in the comparison between group A (treated with ALC) and group B (treated with placebo), significant differences in NH_4^+ were observed in HE1 (−23.4 compared with −3.5; $P < 0.001$) and in HE2 (−28.8 compared with −5.7; $P < 0.001$) (**Table 6**).

Effects of ALC on liver function

At the end of treatment in the group treated with ALC in HE1, we observed significant differences in albumin ($P < 0.05$), AST ($P < 0.05$), and ALT ($P < 0.001$). In HE2, differences were observed in ALT ($P < 0.001$). In the comparison between group A and group B at the end of treatment in HE1, we observed significant differences in prothrombin time (0.4 compared with 1.1; $P < 0.001$), AST (−9.2 compared with −4.6; $P < 0.001$);

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TABLE 4
Comparison of plasma and urinary concentrations of L-carnitine between treatment groups[1]

Variable	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)		P for time[2]	P for group × time[2]
	Before treatment	After 90 d of treatment	Before treatment	After 90 d of treatment		
Free plasma L-carnitine (μmol/L)						
HE1	38.5 ± 3.9	42.3 ± 2.6[3]	38.3 ± 3.8	39 ± 3.5[4]	<0.001	<0.001
HE2	30.8 ± 4.3	36.2 ± 2.8[3]	31.1 ± 5	31.9 ± 4.2[4]	<0.001	<0.001
Plasma SCAC (μmol/L)						
HE1	7.6 ± 0.5	8 ± 0.5[3]	7.2 ± 0.6	7.3 ± 0.6[4]	<0.05	<0.001
HE2	6.5 ± 0.7	7 ± 0.5[3]	6.2 ± 0.5	6.4 ± 0.6[4]	<0.05	<0.001
Plasma LCAC (μmol/L)						
HE1	1.8 ± 0.3	2.1 ± 0.2[3]	2 ± 0.3	2.1 ± 0.4	<0.001	1.000
HE2	1.7 ± 0.4	2 ± 0.3[3]	1.8 ± 0.3	1.9 ± 0.3	<0.001	0.202
Total plasma L-carnitine (μmol/L)						
HE1	48.1 ± 4	52.5 ± 2.8[3]	47.6 ± 4.3	48.5 ± 3.6[4]	<0.001	<0.001
HE2	39.1 ± 4.5	45.3 ± 2.7[3]	39.1 ± 5.0	40.2 ± 4.2[4]	<0.001	<0.001
Free urinary L-carnitine (μmol/L)						
HE1	11.3 ± 0.6	11.6 ± 0.6	11.3 ± 0.9	11.4 ± 0.7	0.054	0.235
HE2	10.9 ± 0.5	11.3 ± 0.4[3]	11.1 ± 0.6	11.3 ± 0.5	<0.001	1.000
Urinary SCAC (μmol/L)						
HE1	10.7 ± 0.5	10.8 ± 0.2	10.8 ± 0.5	11.1 ± 0.4[4]	0.305	<0.001
HE2	11 ± 0.4	11.2 ± 0.4	11.1 ± 0.5	11.4 ± 0.5	0.058	0.092

[1] All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; SCAC, short-chain acylcarnitine; LCAC, long-chain acylcarnitine. There were no significant differences between groups at baseline.
[2] Determined with ANOVA.
[3] Significantly different from before treatment, $P < 0.05$.
[4] Significantly different from ALC treatment, $P < 0.05$.

in HE2, the differences were significant in prothrombin time (0.4 compared with 1.8; $P < 0.001$), bilirubin (−0.2 compared with 0.1; $P < 0.05$), AST (−9.6 compared with −7.9; $P < 0.01$), and ALT (−15.2 compared with −6.2; $P < 0.05$) (Table 6).

Tolerability

Three patients in the ALC group (1 with mild HE and 2 with moderate HE) withdrew from the study because of abdominal pain. One patient in the placebo group withdrew from the study because of headaches. In the placebo group, we observed occasional abdominal pain, cramping, diarrhea, and flatulence. At follow-up 1 mo after treatment ended, 2 patients in the ALC group and 5 patients in the placebo group experienced moderate HE.

DISCUSSION

Fatigue is a multidimensional syndrome and can be described in terms of perceived energy, mental capacity, psychological status, sport, and physical exercise. The suggestion that ammonia accumulation has a significant role in fatigue is not new. It was established that there was an intensity-dependent relation between plasma ammonia concentration and exercise (29). In our study we observed reductions in severity in both physical and mental fatigue and improvements in physical activity and physical function after ALC administration. We also observed an improvement in 7D/PAR, in average daily steps measured by pedometer and in SPPB, whereas poor improvements were recorded in the placebo recipients. Fatigue is a subjective sensation with decreased energy, decreased concentrations, and decreased motivation; it can impair daily functioning and lead to negative effects on quality of life and self-care capabilities (30). Numerous mechanisms and contributory factors have been implicated in fatigue, including *1*) build-up of peripheral toxins and metabolic byproducts and changes in peripheral environment (31, 32), *2*) centrally mediated self regulation (33), *3*) inflammatory cytokine production (34–36), *4*) alterations in neurotransmitter metabolism (37), and *5*) periphery-regulated central drive control (38). The suggested mechanisms include an imbalance in energy metabolism due to increased energy requirements, decreased availability of metabolic substrates, and an abnormal production of substances that impair metabolic homeostasis or normal muscle functioning. The ALC treatment in our study significantly reduced both physical and mental fatigue. L-Carnitine and ALC are often used to foster exercise performance.

There is evidence of a beneficial effect of L-carnitine and ALC supplementation in training competition and recovery from strenuous exercise and in regenerative athletics (39). A great deal of research has investigated the effects of L-carnitine and ALC supplementation on exercise performance—the main premise being that increasing L-carnitine availability would increase fat oxidation during prolonged exercise, spare glycogen stores, and thus delay the onset of fatigue (40). The increase in ALC formation during high-intensity exercise, which occurs to a greater extent in type 1 muscle fibers (41), is directly related to an increase in muscle acetyl-CoA (42, 43), which suggests that the rate of acetyl-CoA formation from pyruvate oxidation, catalyzed by the pyruvate dehydrogenase complex, is in excess of its utilization by the tricarboxylic acid cycle. In our study we observed a significant decrease in serum ammonia concentrations and a significant improvement in mental function in

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TABLE 5
Comparison of evaluated parameters within groups and between groups[1]

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and HE2)			
	Before treatment	90 d after treatment	Before treatment	90 d after treatment	P for time[2]	P for group × time[2]
Physical fatigue score (0–16)						
HE1	11.8 ± 1.8	9.5 ± 2.2[3]	9.9 ± 2.4	9.3 ± 1.4	<0.001	0.675
HE2	10.6 ± 2.3	8.7 ± 1.1[3]	10.4 ± 2.5	8.9 ± 1.7	<0.001	0.591
Mental fatigue score (0–10)						
HE1	7.3 ± 1.5	5.6 ± 1.2[3]	6.1 ± 1.3	6.4 ± 1.5[4]	<0.001	<0.05
HE2	7.1 ± 1.5	6.2 ± 1.1[3]	7.8 ± 1.2	5.8 ± 1.3	<0.05	0.203
Fatigue severity scale (9–63)						
HE1	40.8 ± 4	34.4 ± 2.9[3]	41.8 ± 4.9	44.1 ± 4.5[4]	<0.001	<0.001
HE2	53.6 ± 5	45.5 ± 4.4[3]	54.5 ± 4.9	49.4 ± 4.8[4]	<0.001	<0.001
7D/PAR						
HE1	214.8 ± 24.4	231.9 ± 21.3[3]	205.6 ± 23.6	203.1 ± 15.2[4]	<0.05	<0.001
HE2	166.8 ± 22.4	197.4 ± 18.4[3]	196.6 ± 29.6	205.6 ± 29.1	<0.001	0.197
Pedometer (average daily steps)						
HE1	4902.5 ± 481.5	4996.7 ± 492.9	5006.3 ± 501.6	5020 ± 477.3	0.450	0.852
HE2	3846.5 ± 460	4199.6 ± 364.6[3]	4170.6 ± 449.7	4178 ± 333.1	<0.001	0.812
6MWT						
HE1	372.5 ± 21.4	382 ± 21.6	373 ± 18.8	377 ± 15.4	0.087	0.304
HE2	286 ± 46.7	305.9 ± 34.8	276.9 ± 45.7	279.2 ± 37.2[4]	0.063	<0.05
SPPB						
HE1	7 ± 1	9.1 ± 1.5[3]	7 ± 1.3	7.2 ± 1.1[4]	<0.001	<0.001
HE2	6.3 ± 1.9	8 ± 1.7[3]	7.5 ± 1.7	8 ± 1.5	<0.001	1.000

[1] All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; 7D/PAR, 7-d Physical Activity Recall questionnaire score; 6MWT, 6-min walk test; SPPB, Short Physical Performance Battery. There were no significant differences between groups at baseline.
[2] Determined with ANOVA.
[3] Significantly different from before treatment, $P < 0.05$.
[4] Significantly different from ALC treatment, $P < 0.05$.

patients treated with ALC. Ammonia is a product of the metabolism of nitrogen-containing compounds and is involved in many metabolic reactions. However, ammonia is toxic at elevated concentrations and must be removed from the body (44–46). In patients with HE, brain and muscle cells are involved in the metabolism of ammonia to a greater extent than normal. These "ammonia sinks" use the amino acid glutamate to detoxify ammonia by converting it to glutamate (47). Skeletal muscle metabolizes ammonia in patients with cirrhosis. Loss of lean body mass depletes this ammonia sink and increases the ammonia load to the brain, thereby worsening HE. HE is the result of multiple biochemical influences on central neurotransmitter systems.

In addition to the neurotoxic effects of ammonia, derangements in the γ-aminobutyric acid–ergic (GABA-ergic), serotoninergic, and dopaminergic systems are evident. Reduced detoxification of neurotoxic substances, particularly ammonia, in the cirrhotic liver and subsequently alterations in several neurotransmitter systems and brain edema are supposed to be major factors in the development of HE (48–50). Neurotransmitter systems are affected by increased intracerebral concentrations of ammonia, including the GABA-ergic, glutamatergic, and serotoninergic systems (51–53). Some studies estimated that ≈50% of ammonia may be metabolized in muscle to form glutamine via the glutamine synthetase reaction (54). In animal models of acute and chronic liver failure, hyperammonemia is associated with a rapid increase in glutamine synthetase activity in the skeletal muscle, which results in an increase in the muscle's capacity to remove ammonia (55). In patients with cirrhosis, skeletal muscles may metabolize more ammonia than the cirrhotic liver (56). Previous studies showed that ALC decreases the severity of physical and mental fatigue (57–59). ALC mobilizes acetyl groups and stimulates phospholipid synthesis and increases acetyl-coenzyme A and choline uptake and acetylcholine release (60). It is also involved in the synthesis of glutamate; in fact, the acetyl moiety of ALC is metabolized mainly to glutamate, but also to glutamine, aspartate, and GABA via the tricarboxylic acid cycle. Studies of the role of ALC in aged rat brains showed, in the brain regions with lower amino acid concentrations, that the release of neurotransmitter amino acids is below normal and ALC produces an increase in the extracellular concentration of neurotransmitter glutamate. On the other hand, ALC decreases glutamate dehydrogenase activity in the intrasynaptic mitochondria of the rat brain, which suggests that ALC interferes with glutamate metabolism. The increase in glutamate, caused by elevated plasma ALC concentrations, results in protection against excitotoxic cell death. This is possible through the direct antagonism of glutamate receptors and the activation of GABA receptors that cause neuronal hyperpolarization and therefore resistance to NMDA receptor activation or to inhibition of secondary events. These secondary events could include activation of the mitochondrial

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TABLE 6
Comparison of laboratory values within and between groups[1]

	Group A: ALC (n = 31 HE1 and 30 HE2)		Placebo group (n = 30 HE1 and 30 HE2)		P for time[2]	P for group × time[2]
	Before treatment	90 d after treatment	Before treatment	90 d after treatment		
NH_4^+ (mg/dL)						
HE1	78.3 ± 10.9	54.9 ± 10.1[3]	71.4 ± 9.8	67.9 ± 10.5[4]	<0.001	<0.001
HE2	111.2 ± 14.8	82.4 ± 18.3[3]	99.4 ± 12.9	93.7 ± 11.6[4]	<0.001	<0.001
Albumin (g/dL)						
HE1	3.5 ± 0.3	3.7 ± 0.4[3]	3.5 ± 0.3	3.4 ± 0.3[4]	<0.05	<0.05
HE2	3.5 ± 0.3	3.6 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	0.134	0.058
Prothrombin time (%)						
HE1	74.1 ± 6.8	74.5 ± 5.3	61.6 ± 5.7	62.7 ± 4.8[4]	0.797	<0.001
HE2	65 ± 5.2	65.4 ± 3.9	59.3 ± 5	61.1 ± 4.6[4]	0.735	<0.001
Bilirubin (mg/dL)						
HE1	2.1 ± 0.5	2 ± 0.4	1.7 ± 0.3	1.7 ± 0.2[4]	0.388	<0.001
HE2	2.2 ± 0.6	2 ± 0.5	2.2 ± 0.5	2.3 ± 0.4[4]	0.166	<0.05
AST (IU/L)						
HE1	98.6 ± 12.8	89.4 ± 8.7[3]	105.3 ± 12.4	100.7 ± 13.1[4]	<0.05	<0.001
HE2	124.4 ± 22.4	114.8 ± 17.1	154.9 ± 10.6	147 ± 9.6[4]	0.067	<0.001
ALT (IU/L)						
HE1	111.5 ± 10.7	99.4 ± 7.3[3]	105.2 ± 10.6	92.6 ± 19.5	<0.001	0.075
HE2	140.7 ± 13.8	125.5 ± 7.5[3]	136.8 ± 23.5	130.6 ± 17.2[4]	<0.001	<0.05

[1] All values are means ± SDs. ALC, acetyl-L-carnitine; HE1, patients with mild hepatic encephalopathy; HE2, patients with moderate hepatic encephalopathy; AST, aspartate transaminase; ALT, alanine transaminase. There were no significant differences between groups at baseline.
[2] Determined with ANOVA.
[3] Significantly different from before treatment, $P < 0.05$.
[4] Significantly different from ALC treatment, $P < 0.05$.

permeability transition that can cause the release of mitochondrial cytochrome *c* and stimulation of reactive oxygen species production. These studies showed that the administration of 2 g ALC twice a day attenuated the effect of hyperammonemia.

The major finding and new discovery of the present study was that ALC supplementation can beneficially affect the severity of both physical and mental fatigue. These findings might have been influenced by the limitations in the methods used to assess physical activity. The pedometer might have inaccurately measured the magnitude of physical activity before and after treatment. For example, the pedometer might not have accurately assessed activities other than level walking, the positioning of the pedometer could have affected the accuracy of the measurement, and subjects were required to accurately self-report their daily steps to the investigators (61). It has also been shown that cirrhosis individuals inaccurately report their physical activity, which could have contributed to the patterns observed in this study (62). Therefore, the use of objective monitoring of physical activity should be considered for future studies. With regard to the whole study population, a clear treatment effect in favor of ALC was shown regarding the improvement in EEG grading, fatigue severity, and physical activity. Accordingly, a superiority of ALC in comparison with placebo was shown in the subgroups with HE2. Otherwise, it could be shown that the response to ALC in patients with HE1 is smaller than that in those patients with HE2. On the basis of the ORs, it was observed that the greater the initial mental state gradation of HE, the greater the effect of ALC on EEG grading and fatigue severity.

In conclusion, the administration of ALC in compensated patients with cirrhosis could enhance the tolerance to protein load and low ammonia concentrations and improve neurologic symptoms in patients with HE and was at least as useful as placebo in the long-term treatment of both chronic grade 1 and grade 2 HE. The patients with HE treated with ALC showed a decrease in the severity of both mental and physical fatigue, an increase in physical activity, and an improvement in daily functioning. Treatment with ALC may lead to a positive spiral: an improvement in physical activity that leads to a reduction in the severity of fatigue, which leads to further activity (63). The role of ammonia in the neuromuscular activity of patients with HE remains to be determined in future studies.

The authors' responsibilities were as follows—Mariano Malaguarnera and FG: contributed to the study design, data analysis, and drafting of the manuscript; GP, RB, and LR: contributed to the neurophysiologic assessment of the patients; MV and MG: helped with the statistical analysis and data interpretation; and Michele Malaguarnera, GLV, and FG: contributed to the laboratory assessment of the patients. The authors disclosed no conflicts of interest.

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