

L-Carnitine Supplementation to Diet: A New Tool in Treatment of Nonalcoholic Steatohepatitis—A Randomized and Controlled Clinical Trial

Mariano Malaguarnera, AP¹, Maria Pia Gargante, MD¹, Cristina Russo, MD¹, Tijana Antic, MD¹, Marco Vacante, MD¹, Michele Malaguarnera, MD², Teresio Avitabile³, Giovanni Li Volti, AP² and Fabio Galvano, AP²

- OBJECTIVES:** Nonalcoholic steatohepatitis (NASH) is a known metabolic disorder of the liver. No treatment has been conclusively shown to improve NASH or prevent disease progression. The function of L-carnitine to modulate lipid profile, glucose metabolism, oxidative stress, and inflammatory responses has been shown. The aim of this study was to evaluate the effects of L-carnitine's supplementation on regression of NASH.
- METHODS:** In patients with NASH and control subjects, we randomly dispensed one 1-g L-carnitine tablet after breakfast plus diet and one 1 g tablet after dinner plus diet for 24 weeks or diet alone at the same dosage and regimen. We evaluated liver enzymes, lipid profile, fasting plasma glucose, C-reactive protein (CRP), tumor necrosis factor (TNF)- α , homeostasis model assessment (HOMA)-IR, body mass index, and histological scores.
- RESULTS:** At the end of the study, L-carnitine-treated patients showed significant improvements in the following parameters: aspartate aminotransferase ($P=0.000$), alanine aminotransferase (ALT) ($P=0.000$), γ -glutamyl-transpeptidase (γ -GT) ($P=0.000$), total cholesterol ($P=0.000$), low-density lipoprotein (LDL) ($P=0.000$), high-density lipoprotein (HDL) ($P=0.000$), triglycerides ($P=0.000$), glucose ($P=0.000$), HOMA-IR ($P=0.000$), CRP ($P=0.000$), TNF- α ($P=0.000$), and histological scores ($P=0.000$).
- CONCLUSIONS:** L-carnitine supplementation to diet is useful for reducing TNF- α and CRP, and for improving liver function, glucose plasma level, lipid profile, HOMA-IR, and histological manifestations of NASH.

Am J Gastroenterol 2010; 105:1338–1345; doi:10.1038/ajg.2009.719; published online 12 January 2010

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is the most common cause of chronic liver disease in the western countries. The prevalence is between 10% and 24% in the general population and reaches 75% in the obese groups (1,2). The pathogenesis of NASH is associated with disorders of energy metabolism, including obesity, insulin resistance, and dyslipidemia. The real mechanisms leading to NASH are still unclear, but nutritional, metabolic, genetic, viral, and other factors cause or contribute to fatty liver disease (3–5). The existing model that explains the pathogenesis of NASH is the “two-hit” hypothesis, first proposed by Day and James (6). According to this hypothesis, steatosis represents the

“first hit,” which increases the vulnerability of the liver to various “second hits” that in turn lead to the inflammation, fibrosis, and cellular death (6). Several factors have been suggested to constitute the second hit(s), such as oxidative stress, pro-inflammatory cytokines, and gut-derived bacterial endotoxin (6). It was observed that NASH is associated with a more atherogenic lipid profile, including hypertriglyceridemia, a higher plasma concentration of very low-density lipoprotein (VLDL) and LDL that are larger in size, and with lower levels of high-density lipoprotein (HDL) (7,8). The function of the lipid was highlighted when Unger and Orci (9) introduced the concept of lipoapoptosis according to which overaccumulation of lipids in nonadipose tissues leads to cell dysfunction and death. Given that, the

¹Department of Senescence, Urological and Neurological Sciences, University of Catania, Catania, Italy; ²Department of Biological Chemistry, Medical Chemistry and Molecular Biology University of Catania, Catania, Italy; ³Department of Ophthalmology, University of Catania, Catania, Italy. **Correspondence:** Mariano Malaguarnera, AP, Department of Senescence, Urological and Neurological Sciences, University of Catania, Ospedale Cannizzaro, Viale Messina, Catania 829-95125, Italy. E-mail: malaguar@unict.it

Received 10 September 2009; accepted 2 December 2009

presence of increased circulating and/or hepatic saturated fatty acids might promote the development and progression of liver damages activating apoptosis (10,11). Moreover, in the liver, the increase of fatty acids synthesis associated with the reduction of their delivery from hepatocytes by VLDL because of degradation of apolipoprotein B100 causes the unbalance of hepatic fat turnover resulting in steatosis (12). Fatty acids are a source of oxidative stress and damage of mitochondria with increased β -oxidation and raising levels of reactive oxygen species (ROS) (13). Recently, it has been hypothesized that the L-carnitine, a quaternary amine, could improve the outcome of NASH, because it reduces lipid levels, limits oxidative stress, and modulates inflammatory responses (14). It performs a number of essential intracellular and metabolic functions, such as fatty acid transport, detoxification of potentially toxic metabolites, regulation of the mitochondrial acyl-Co A/CoA ratio, and stabilization of cell membranes. It has a pivotal role in the transport of long-chain fatty acids across the inner mitochondrial membrane. L-carnitine facilitates the elimination of short- and medium-chain fatty acids accumulating in mitochondria as a result of normal or abnormal metabolism (15). The aim of this study was to evaluate whether L-carnitine treatment could determine histological changes at liver biopsy and modify humoral parameters after a 24-week treatment in patients with NASH.

METHODS

Study design

This was a randomized, double-blind, placebo-controlled study. The study was conducted between January 2004 and December 2006, and the study participants were recruited from Cannizzaro Hospital, Catania, Italy.

This study was designed and conducted in compliance with the ethical principles of Good Clinical Practice Guidelines and the Declaration of Helsinki (16). The study protocol was approved by the research ethics committee of Cannizzaro Hospital, Catania, Italy. Informed consent was obtained from patients before any study procedures were initiated. Eighty patients with a clinical and pathologic diagnosis of NASH were enrolled in the study. Seventy-four patients (40 men and 34 women; age 28–60 years, mean age 47.6 years) were randomly assigned by a computer-generated randomization schedule to receive a 24-week supply of either L-carnitine or placebo. Thirty-eight patients were allocated to placebo group and 36 were allocated to L-carnitine group. None of the patients withdrew from the planned treatment (Figure 1). The treatment period was 24 weeks. The measurements were made every month, both for efficacy tests and tolerability.

Patients

Laboratory features of controls and patients included in this study are similar and they are summarized in Tables 1 and 2. The data included subjects who had, for at least 6 months, abnormal serum aminotransferase levels that were not related to other causes of liver disease. All patients underwent percutaneous liver

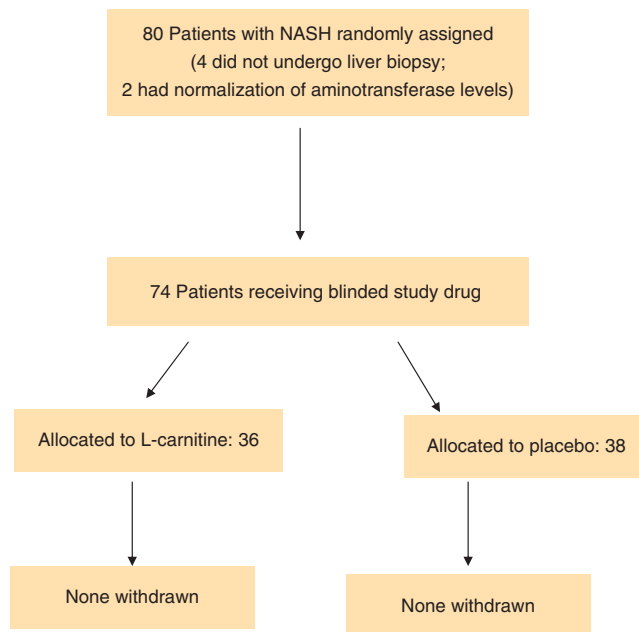


Figure 1. Trial profile of L-carnitine treatment. NASH, nonalcoholic steatohepatitis.

biopsy, and the diagnosis of NASH was established by the presence of pericentral macrovesicular steatosis, ballooning degeneration of the liver cells with or without Mallory bodies or fibrosis, and lobular and portal inflammation in the absence of other causes of liver disease (viral, drugs, toxin, autoimmune, metabolic). Significant alcohol consumption was a criteria of exclusion (>10 g per day for females and >20 g per day for males). Other causes of exclusion were hereditary hemochromatosis, α -1 antitrypsin deficiency, Wilson's disease prior surgical procedures such as jejunioileal or jejunocolic bypass, gastroplast, total parenteral nutrition in the past 6 months, pregnancy, use of drugs such as calcium channel blockers, high dose of synthetic estrogens, methotrexate, amiodarone steroids, chloroquine, a history of treatment with lipid-lowering agents, a history of hypothyroidism, or Cushing syndrome. After a 4-week washout period, 74 patients were asked to follow the National Cholesterol Education Program Adult Treatment Panel III therapeutic lifestyle-change diet; then eligible patients were randomized to receive L-carnitine or placebo (17). A group received L-carnitine 2 g per day divided into two equal doses of one 1 g tablet after breakfast and one 1 g tablet after dinner for 24 weeks (L-carnitine, Sigma Tau, Pomezia, Italy). The other group received placebo according to the same regimen and for the same duration.

Prerandomization phase

The subjects were required to document all caloric intake with the use of a diary, completed every 2 days. This prerandomization period was designed to nullify the effects of dietary changes on metabolic markers. During the initial 4-week phase, subjects were instructed by a dietitian to follow an ad libitum diet as classified by the National Cholesterol Education Program (17).

Table 1. Baseline characteristics and laboratory parameters at enrollment

| Laboratory parameter | Normal values | Diet | L-carnitine + diet | P value |
|--------------------------|---------------|------------|--------------------|---------|
| No. | | 38 | 36 | NS |
| Age (years) | | 47.8±5.8 | 47.9±5.4 | NS |
| Gender (M/F) | | 20/18 | 20/16 | NS |
| BMI (kg/m ²) | | 26.5±3.8 | 26.6±3.7 | NS |
| Heart rate (b.p.m.) | | 86 ±10 | 84 ±11 | NS |
| SBP (mm Hg) | | 140±11 | 141±12 | NS |
| DBP (mm Hg) | | 86±5 | 84±7 | NS |
| Glucose (mmol/l) | 5.0–7.0 | 6.10±0.67 | 6.14±0.69 | NS |
| Insulin (mIU/l) | 5.0–20.0 | 17.2±5.6 | 17.3±5.5 | NS |
| C-peptide (mmol/l) | 0.11±0.61 | 0.98±0.54 | 0.97±0.61 | NS |
| Albumin (g/l) | 34–50 | 48.2±5.4 | 47.8±5.6 | NS |
| AST (IU/l) | 10–45 | 135.4±15.1 | 132.8±14.7 | NS |
| ALT (IU/l) | 10–43 | 120.2±12.8 | 125.7±12.9 | NS |
| ALP (IU/l) | 80–260 | 196±55 | 194±60 | NS |
| Prothrombin time (%) | 85–100 | 94±9 | 95±10 | NS |
| γ-GT (IU/l) | 1–36 | 120.8±37.2 | 121.1±37.4 | NS |
| Cholesterol (mmol/l) | 5.17–6.18 | 6.24±0.66 | 6.28±0.64 | NS |
| HDL cholesterol (mmol/l) | 1.04–1.29 | 0.95±0.10 | 0.92±0.11 | NS |
| LDL cholesterol (mmol/l) | 1.55–4.4 | 4.68±0.82 | 4.74±0.83 | NS |
| Triglycerides (mmol/l) | 0.70–2.1 | 3.08±0.81 | 3.10±0.90 | NS |
| CRP (mg/dl) | 0.2–1.0 | 8.8±3.2 | 9.0±3.4 | NS |
| TNF-α (pg/ml) | 0.4–2.5 | 3.40±0.25 | 3.51±0.30 | NS |
| HOMA-IR | | 4.66±0.74 | 4.72±0.78 | NS |

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; γ-GT, γ-glutamyl-transpeptidase; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; SBP, systolic blood pressure; TNF-α, tumor necrosis factor-α; NS, not significant.

Patients were checked by a dietician every month; at each visit, the dietician 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 the groups were given the same 1,600-calorie diet and were prescribed an exercise plan. Both groups had a 30-min home-based whole-body stretching routine to perform three times per week. Subjects received one supervised stretching session at treatment initiation, a booklet detailing the stretches, and were unsupervised thereafter. All individuals were informed that the research hypothesis was that regular stretching could reduce inflammation and assist in the preferential reduction of adiposity from the liver and viscera. Subjects underwent weekly visits throughout the treatment period to assess the adherence to the study protocol, to measure blood pressure, and to record adverse events.

Randomization phase

Throughout the trial, L-carnitine was supplied in vials with 2 g carnitine taken orally twice a day. All drugs and placebos were identical in appearance, and neither investigators nor patients

were informed of the selected agent until the end of the study phase. Dosing instructions were provided with each patient pack. All trial medications were 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 drug was administered at the lowest possible therapeutic dosage and, as far as possible, was not changed.

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 aspartate aminotransferase, alanine aminotransferase (ALT), γ-glutamyl-transpeptidase (γ-GT), albumin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, insulin, C-peptide, C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), alkaline phosphatase, and prothrombin time. Measurements were made at the beginning and at the end of the study period. Data were collected in the morning, after an overnight fast.

Table 2. Laboratory parameters in patients treated with placebo plus diet or L-carnitine plus diet before and after 24 weeks of treatment^a

| | Group A (placebo + diet) n=38 | | Group B (L-carnitine + diet) n=36 | |
|----------------------------|-------------------------------|----------------|-----------------------------------|----------------|
| | Before treatment | After 24 weeks | Before treatment | After 24 weeks |
| BMI (kg/m ²) | 26.5±3.8 | 25.4±3.9* | 26.6±3.7 | 25.3±3.5***†† |
| AST (IU/l) | 124.2±12.8 | 78.1±21.4***† | 128.1±13.9 | 56.4±18.2***† |
| ALT (IU/l) | 112.8±13.1 | 75.4±13.9***† | 110.2±15.6 | 51.8±16.4***† |
| γ-GT (IU/l) | 98.2±18.2 | 77.8±19.4***† | 104.1±17.2 | 66.5±15.4***† |
| Albumin (g/l) | 48±5.1 | 48.7±5.4***† | 47.8±5.4 | 48.1±5.6***† |
| Total cholesterol (mmol/l) | 6.04±0.91 | 5.88±0.98*† | 6.09±0.94 | 4.78±0.82***† |
| HDL (mmol/l) | 0.98±0.12 | 1.10±0.14***†† | 0.96±0.14 | 1.16±0.12***†† |
| LDL (mmol/l) | 4.45±0.87 | 4.24±0.88*† | 4.52±0.95 | 3.15±0.66***† |
| Triglycerides (mmol/l) | 3.04±0.96 | 2.68±0.92***† | 3.07±0.88 | 2.34±0.66***†† |
| Glucose (mmol/l) | 6.04±0.66 | 5.97±0.71*† | 6.12±0.69 | 5.32±0.55***† |
| Insulin (mIU/l) | 16.8±5.4 | 15.2±5.2***† | 17.1±5.6 | 13.8±5.0***† |
| C-peptide (mmol/l) | 0.96±0.4 | 0.94±0.41***† | 0.98±0.41 | 0.93±0.62***† |
| HOMA-IR | 4.5±0.67 | 4.03±0.71**† | 4.65±0.70 | 3.26±0.56***† |
| CRP (mg/dl) | 8.7±3.4 | 7.4±3.2***† | 9.1±3.2 | 5.2±3.1***† |
| TNF-α (pg/ml) | 1.38±0.22 | 1.30±0.21*† | 1.44±0.28 | 1.08±0.15***† |

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; γ-GT, γ-glutamyl-transpeptidase; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; TNF-α, tumor necrosis factor-α; NS, not significant.

^aAll values are x ± s.d.

Comparison within group A and within group B according to the values before the treatment.

*P = NS; **P < 0.05; ***P < 0.001.

Comparison between groups A and B after 24 weeks of treatment.

†P < 0.001; ††P < 0.05; †††NS.

Tolerability assessment

Liver biopsy was made at the beginning and then repeated at the end of the treatment period after providing explicit ethics committee approval. In fact, liver biopsy samples remain the only way to establish a definitive diagnosis of NASH and determine the stage of hepatic fibrosis, thereby also providing prognostic information. Body mass index was calculated as weight/height² (kg/m²). Laboratory assessments were monitored at baseline and monthly, until the end of the trial. These data included hemochrome, glycemia, creatinemia, and blood urea. After providing informed consent, each subject in the two groups underwent ultrasonography examination of the liver. Electrocardiogram and blood pressure were monitored with the use of standard techniques.

Clinical laboratory tests

Blood samples were obtained after the patients had fasted for 12h overnight. Venous blood samples were taken from all patients between 8 AM and 10 AM. Plasma was obtained from the blood samples by the addition of ethylene diaminetetraacetic acid and centrifugation at 3,000g for 15 min at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at -80°C. The fasting plasma glucose levels were assayed using the glucose-oxidase method with intra- and inter-assay coefficients of variation (CV) of 0.8% and 2.4%, respectively. The total cholesterol and triglycerides were determined using fully enzymatic techniques on a

clinical chemistry analyzer whose intra- and inter-assay CVs were 1.2% and 2.3%, respectively, for the total cholesterol measurement and 1.1% and 2.4%, respectively, for the TG measurement. The HDL cholesterol level was measured after precipitation of plasma apo-B containing lipoproteins with phosphotungstic acid. The intra- and inter-assay CVs were 1.0% and 2.0%, respectively. The LDL cholesterol level was calculated using the Friedewald formula (18). Insulin was measured using a two-site immunoenzymatic assay performed on the Access automated immunoassay system. Intra-assay CVs are 2.1% at 6.70 μU/ml and 2.5% at 116 μU/ml. Inter-assay CVs are 3.8% at 12.7 μU/ml, 4.1% at 48.8 μU/ml, and 4.5% at 121 μU/ml. C-peptide was measured by a direct, double antibody sequential radioimmunoassay. Inter-assay CVs are 4.9% at 0.43 and 1.75% at 4.36 nmol/l. A measure of insulin resistance was performed with homeostasis model assessment (HOMA). The homeostatic model assessment (HOMA-IR) was calculated using the formula IR = insulin × glucose / 22.5. Higher values of HOMA-IR indicate more insulin resistance. Serum-sensitive CRP was measured at baseline and halfway through the intervention by a particle-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The serum TNF-α was analyzed with the BD Cytometric-Bead Array Kid (BD Biosciences, San Diego, CA) at baseline and at the end of intervention. Samples were processed in duplicate. Laboratory evaluation included serum liver tests (total protein, albumin,

aspartate transaminase, alanine transaminase (ALT), γ -GT, alkaline phosphatase, and total serum bilirubin), hepatitis B serology (hepatitis B surface antigen, and antibody to hepatitis B surface antigen, antibody to hepatitis B core antigen), antibody to hepatitis C virus, hepatitis C RNA polymerase chain reaction, autoantibodies (antinuclear antibody, antismooth muscle antibody, and antimitochondrial antibody), and iron profile (serum iron, transferrin saturation, and ferritin). All values were determined following a 12-h fasting period by the hospital clinical laboratory.

Ultrasonography test

To perform ultrasonographic scan, we used a real-time machine (Logiq 500, General Electrics) with a linear 3.5-MHz transducer (Pie Medical Scanner 150). This technique is reported to have a high sensitivity and specificity for the diagnosis of fatty liver, defined as the presence of fat in >30% of each hepatic lobule, when a combination of the following four parameters are used: (1) diffuse hyperechoic echotexture (bright liver), (2) increased liver echotexture compared with the kidneys, (3) vascular blurring, and (4) deep attenuation.

Histological analysis

Liver biopsies were obtained using a 16-gauge Klatskin needle. A liver specimen of 15 mm with at least 10 portal tracts was considered adequate for evaluation. After completion of the study, all liver biopsy samples were coded and read by a hepatic pathologist without the knowledge of the patient or the sequence of the biopsy. Six histological features of NASH were scored semi-quantitatively from 0 to 4, including steatosis, acinar zone 3 hepatocellular injury (ballooning degeneration), parenchymal inflammation, portal inflammation, perisinusoidal fibrosis, and Mallory bodies. Ubiquitin immunostaining was used to help identify Mallory bodies. The primary outcome measure for this study was improvement in liver histology as assessed by the NASH-activity index. The NASH-activity index was defined by the sum of scores for steatosis, parenchymal inflammation, and hepatocellular injury, and thus ranged from 0 to 12. Improvement was defined as a decrease in the NASH-activity index of at least three points with improvements of at least one point for each of the three features (19).

Statistical analysis

The data were analyzed using the Statistical Analysis System software version 6.11 (SAS Institute, Cary, NC). The differences between the means were evaluated by using analysis of variance or paired *t*-tests, where appropriate. All results were expressed as mean \pm s.d. unless otherwise mentioned. Pearson's correlation coefficient was used for correlation analysis between variables. *P* values <0.05 were considered significant. Tukey's *post hoc* tests were used to assess the differences between the treatment groups. Data were further analyzed with a Bonferroni adjusted *t*-test for multiple comparisons. The Mann-Whitney *U*-test was used to compare nonparametric data. The primary population for statistical analysis was an intention-to-treat population of all randomly assigned subjects.

RESULTS

The patients were enrolled between January 2001 and March 2003. Eighty patients suspected of having NASH were evaluated and 74 patients were enrolled in the study. Reasons for exclusion included patient's unwillingness to undergo liver biopsy (*n*=4) and normalization of aminotransferase levels during the prerandomization phase (*n*=2) (Figure 1).

Biochemical responses

Effects of L-carnitine on liver enzymes. At the end of the evaluation (24 weeks), subjects treated with L-carnitine plus diet compared with placebo plus diet group showed significant differences in aspartate aminotransferase (*P*=0.000), ALT (*P*=0.000), γ -GT (*P*=0.007) (Table 2).

Effects of L-carnitine on lipid profile. At 24 weeks, L-carnitine plus diet-treated patients compared with placebo plus diet group showed significant difference in total cholesterol (*P*=0.000) and LDL cholesterol (*P*=0.000) (Table 2).

Effects of L-carnitine on glycometabolic profile. At the end of the study period, we observed that L-carnitine plus diet compared with placebo plus diet showed a significant decrease in plasma glucose level (*P*=0.000) and HOMA-IR (*P*=0.000) (Table 2).

Effects of L-carnitine on inflammation factors. At the end of 24 weeks, L-carnitine plus diet compared with placebo plus diet group showed significant decreases in CRP (*P*=0.004) and TNF- α (*P*=0.000) (Table 2).

Histological responses

Repeat liver biopsies were available on all 36 patients treated with L-carnitine. Each of the component features of the NASH-activity index (steatosis, parenchymal inflammation, and hepatocellular injury) improved significantly, as did fibrosis and Mallory bodies. Thirty-one patients (86%) had improvement in fibrosis scores (17 patients had one level reduction, 12 had two levels reduction, and 2 had three levels reduction of fibrosis score). Fibrosis scores were unchanged in five patients (14%).

Overall, the mean NASH-activity score decreased from 9.42 (range, 8–11) at baseline to 3.19 (range, 1–8) at 24 weeks. The NASH-activity score decreased by at least two points in all patients. A histological response was defined as a reduction in the NASH-activity index by three points or more with improvements of at least one point each in steatosis, parenchymal inflammation, and hepatocellular injury. Using this strict definition, 35 patients (97%) had a histological response (Table 3).

Tolerability. Both L-carnitine and placebo were well tolerated in 100% of patients. In the group treated with L-carnitine, one patient reported nausea, two moderate headache, and two abdominal pain. In the placebo group, two patients reported diarrhea, one moderate headache, and two fatigue. Patients were evaluated at baseline, after 1 month, and for next 6 months every 4 weeks.

Table 3. Histological data in patients treated with placebo plus diet or L-carnitine plus diet before and after 24 weeks of treatment^a

| Parameter (0–4) | Group A (placebo + diet) n=38 | | Group B (L-carnitine + diet) n=36 | |
|-----------------------------------|-------------------------------|----------------|-----------------------------------|----------------|
| | Before treatment | After 24 weeks | Before treatment | After 24 weeks |
| Steatosis | 2.79±0.47 | 1.68±0.76***† | 3.22±0.63 | 0.94±0.88***† |
| Hepatocellular injury | 2.66±0.47 | 1.47±0.64***† | 3.00±0.33 | 1.05±0.74***† |
| Parenchymal inflammation | 2.76±0.43 | 1.42±0.63***† | 3.22±0.48 | 1.94±0.7***† |
| Portal inflammation | 2.89±0.38 | 1.82±0.68***† | 2.77±0.48 | 1.28±0.80***† |
| Fibrosis | 2.74±0.44 | 1.89±0.72***† | 2.86±0.53 | 1.55±0.72***† |
| Mallory bodies | 2.74±0.50 | 1.53±0.72***†† | 2.94±0.47 | 1.42±0.68***†† |
| NASH activity (0–12) ^b | 8.21±0.86 | 4.58±1.31***† | 9.42±0.76 | 3.19±1.71***† |

NASH, nonalcoholic steatohepatitis.

^aSum of steatosis, hepatocellular injury, and parenchymal inflammation.^bAll values are $\bar{x} \pm \text{s.d.}$

Comparison within group A and within group B according to the values before the treatment.

P* = NS; *P* < 0.05; ****P* < 0.001.

Comparison between groups A and B after 24 weeks of treatment.

†*P* < 0.001; ††*P* < 0.05; †††NS.

DISCUSSION

In this study, we found that the patients with NASH who were treated with L-carnitine compared with patients who were treated with placebo have an improvement in histological findings of the liver.

We registered a decrease in hepatic inflammation and fibrosis supported by reduction of some inflammatory indexes such as CRP and TNF- α . Probably, TNF- α might have a pivotal role given that mitochondrial dysfunction was associated with increased serum TNF- α levels. Naturally, an improvement in lipid profile and also a better insulin sensitivity and lower values of fasting plasma glucose have a close relationship with a reduction of steatosis such as many studies reported (20,21).

The study of pathophysiologic process and molecular mechanisms of NASH is limited by the lack of appropriate animal models, but we can focus our attention on mitochondrial β -oxidation (causing steatosis) and respiration (causing increased formation of ROS and adenosine triphosphate depletion) through inhibition of carnitine palmitoyl transferase I and acyl-coenzyme A, respectively (22).

L-carnitine is an essential factor in the production of acetyl-CoA. It regulates the turnover of the fatty acids into phospholipids membranes, a process known as the deacylation–reacylation cycle of phospholipids membranes. L-carnitine is suggested to act as CoA buffer, maintaining the acyl Co A/CoA ratio in cells and exerts a function in several metabolic processes. The transport of acyl-CoA across the inner mitochondrial membrane to the matrix determine a reduced availability of CoA in the matrix and a decrease of CoA-SH. It determines a parallel increase in the acyl coA/CoASH ratio, which inhibits the mitochondrial dehydrogenases; consequently, not only the oxidation of fatty acids but also the utilization of carbohydrates becomes impaired (23).

Various studies have indicated that the risk factors implicated in the development of NASH include insulin resistance, oxidative stress, stellate cell activation, apoptosis, cytokine, and adipokine pathways (4).

L-carnitine could also have beneficial effects on the mitochondrial respiratory chain. Several studies on aging showed that L-carnitine increases the mitochondrial content of cardiolipin reducing the mitochondrial impairment of electron transfer in liver (24). In addition, L-carnitine has some antioxidant and antiapoptotic properties (25). The mechanisms whereby L-carnitine could mediate its antioxidant action is still unclear, but several studies pointed to increased levels of different antioxidant enzymes (e.g., SOD, catalase, glutathione peroxidase) and vitamins (e.g., vitamins C and E) (26). Finally, L-carnitine could exert its antiapoptotic effects by decreasing ROS production, removing toxic fatty acid derivatives, and reducing generation of ceramides (25).

In our study, L-carnitine decreases plasma glucose level and insulin resistance. Insulin resistance is one of the characteristics of the NASH and the metabolic syndrome. However, few studies investigated the effects of carnitine therapy on insulin resistance (27,28). L-carnitine is reported to control hyperglycaemia and improve insulin sensitivity (29) and also increase the peripheral glucose utilization (30) in the insulin resistance patients (31). Deficiency of L-carnitine has been reported in type 2 diabetic women with complication (32), in children with type 1 diabetes (33), in experimental diabetic neuropathy (34), and in streptozotocin-diabetic rats (35).

Moreover, it is well known that amplification of fatty acid esterification pathway and triglycerides formation could be implicated in hepatic insulin resistance (36).

Stored free fatty acid (FFA) can be mobilized from adipose tissue through lipolysis. This process is headed by glucagon, insulin resistance, sudden weight loss or starvation, glucocorticosteroids, leptin, and TNF- α (37). In the patients treated with L-carnitine, we observed a decrease in total and LDL cholesterol and in triglycerides. L-carnitine binds to fatty acyl-CoA and regulates their transport into the mitochondrial matrix for β -oxidation. L-carnitine deficiency causes reduced oxidation of FFA and accumulation of long-chain fatty acyl-CoA and diabetic complications (33). Metabolism of FFA would be diverted toward esterification pathway rather

than oxidation leading to accumulation of diacyl glycerol, and triglyceride. FFA has diabetogenic effects in the liver by having an influence on hepatic glycogenolysis, breakdown of hepatic autoregulation to glycogen deposition, and insulin resistance. FFA also decreases insulin biosynthesis, alters bioinsulin processing, and decreases insulin gene transcription (38,39).

L-carnitine administration in rodents decreases liver triglycerides and hepatic steatosis after administration of a high fat diet, after total parenteral nutrition, or after alcohol intoxication (40–43). Interestingly, in one study dealing with ethanol-induced liver damage, L-carnitine even reduced hepatic inflammation and plasma levels of ALT and TNF- α (23).

One study showed no improvement in transaminase levels, plasma FFA levels, plasma triglyceride levels, or the grade of hepatic steatosis by histological examination (44). Previous studies carried out in chronic hepatitis patients treated with α interferon and ribavirin, L-carnitine treatment showed a reduction of steatosis, fibrosis, and hepatic inflammation (14).

In our study, L-carnitine reduces CRP and TNF- α levels with a huge benefit for patients. Previous data regarding the effect of carnitine in patients with elevated CRP levels showed big benefits too.

Although L-carnitine supplementation improves liver biochemistry, metabolic studies in humans did not show any beneficial effect with the use of L-carnitine supplementation during total parenteral nutrition or on the rates of fatty acid oxidation (45,46).

We highlighted that oxidation of FFAs is the most important cellular source of ROS; on the other hand, FFA are a normal and important compound of our body synthesized in the liver when necessary. Nevertheless, when the liver is overloaded with FFA, it becomes weak to adequately secrete them into circulation; it determines an overload system with synthesis and accumulation of triglycerides in the liver resulting in steatosis (47). Given the potential role of oxidative stress in the pathogenesis of NASH, investigators have focused on the use of antioxidants to protect cellular structures against damage from oxygen free radicals and from reactive products of lipid peroxidation.

In our study, we noted that L-carnitine supplementation induces regression of NASH even if both plasma and hepatic carnitine levels have been shown to be normal in subjects with NASH. Moreover, we noted a decrease of TNF- α , CRP, glucose plasma levels, and improvement of lipid profile. The real mechanism underlying this is not clear, but we can assume that L-carnitine can interfere with processes involved in β -oxidation and accumulation of lipotoxic metabolites that might contribute to mitochondrial dysfunction and insulin resistance. L-carnitine could act through mechanisms that are independent of the putative detoxifying role.

In future studies, it will be important to examine the relationship between circulating and intrahepatic fatty acid composition, liver damage and antioxidant therapy in patients with NASH.

ACKNOWLEDGMENTS

This clinical trial was supported by a grant from MURST (Ministero dell'Università e Ricerca Scientifica e Tecnologica).

CONFLICT OF INTEREST

Guarantor of the article: Mariano Malaguarnera, AP.

Specific author contributions: Contributed to the study design, data analysis, and the drafting of the manuscript: Mariano Malaguarnera; contributed to enrollment of patients and data interpretation: Maria Pia Gargante, Cristina Russo, Marco Vacante, Tijana Antic; helped with statistical analysis, data interpretation, and data analysis: Michele Malaguarnera, Fabio Galvano, and Giovanni Li Volti; contributed to evaluation of retinopathy: Teresio Avitabile.

Financial support: None.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Nonalcoholic steatohepatitis (NASH) treatment is needed to prevent advanced chronic liver disease related to insulin resistance.
- ✓ Although many treatment modalities have been used in patients with NASH, none have been convincingly shown to be effective.
- ✓ Weight reduction and increased physical exertion are effective methods of improving insulin resistance.

WHAT IS NEW HERE

- ✓ L-carnitine is effective in reducing total cholesterol, oxidized low-density lipoprotein cholesterol, and triglycerides, and in improving insulin resistance.
- ✓ L-carnitine treatment and lifestyle changes, including weight loss and exercise, can represent therapeutic options in NASH.

REFERENCES

1. Ludwig J, McGill DB, Lindor KD. Review: nonalcoholic steatohepatitis. *J Gastroenterol Hepatol* 1997;12:398–403.
2. Clark JM, Diehl AM. Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. *JAMA* 2003;289:3000–4.
3. Hegazi RA, Sutton-Tyrrell K, Evans RW *et al.* Relationship of adiposity to subclinical atherosclerosis in obese patients with type 2 diabetes. *Obes Res* 2003;11:1597–605.
4. Malaguarnera L, Rosa MD, Zambito AM *et al.* Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. *Am J Gastroenterol* 2006;101:2060–9.
5. Malaguarnera L, Di Rosa M, Zambito AM *et al.* Chitotriosidase gene expression in Kupffer cells from patients with non-alcoholic fatty liver disease. *Gut* 2006;55:1313–20.
6. Day CP, James O. Steatohepatitis: a tale of two “hits”? *Gastroenterology* 1998;114:842–5.
7. Malaguarnera M, Vacante M, Motta M *et al.* Effect of L-carnitine on the size of LDL particles in the type 2 diabetic patients treated with simvastatin. *Metabolism* 2009;58:1618–23.
8. Galvano F, Li Volti G, Malaguarnera M *et al.* Effects of simvastatin and carnitine vs. simvastatin on lipoprotein(a) and apoprotein(a) in type II diabetes mellitus. *Expert Opin Pharmacother* 2009;10:1–8.
9. Unger RH, Orci L. Lipoapoptosis: its mechanism and its diseases. *Biochim Biophys Acta* 2002;1585:202–12.
10. Targher G, Bertolini L, Poli F *et al.* Nonalcoholic Fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes* 2005;54:3541–6.
11. Shimabukuro M, Zhou YT, Levi M *et al.* Fatty acid-induced B cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci USA* 1998;95:2498–502.
12. Sakata N, Phillips TE, Dixon JL. Distribution, transport, and degradation of apolipoprotein B-100 in HepG2 cells. *J Lipid Res* 2001;42:1947–58.

13. De Almeida IT, Cortez-Pinto H, Fidalgo G *et al*. Plasma total and free fatty acids composition in human non-alcoholic steatohepatitis. *Clin Nutr* 2002;21:219–23.
14. Romano M, Vacante M, Cristaldi E *et al*. L-carnitine treatment reduces steatosis in patients with chronic hepatitis C treated with alpha-interferon and ribavirin. *Dig Dis Sci* 2008;53:1114–21.
15. Walter JH. L-Carnitine. *Arch Dis Child* 1996;74:475–8.
16. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *JAMA* 1997;277:925–6.
17. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
19. Kleiner DE, Brunt EM, Van Natta M, *et al*. Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–21.
20. Li Z, Peraldi P, Yang S *et al*. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003;37:343–50.
21. Ridker PM. Should statin therapy be considered for patients with elevated C-reactive protein? The need for a definitive clinical trial. *Eur Heart J* 2001;22:2135–7.
22. Benzie IF. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *Int J Food Sci Nutr* 1996;47:233–61.
23. Malaguarnera M, Vacante M, Avitabile T *et al*. supplementation reduces oxidized LDL cholesterol in patients with diabetes. *Am J Clin Nutr* 2009;89:71–6.
24. Hagen TM, Liu J, Lykkesfeldt J *et al*. Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress. *Proc Natl Acad Sci USA* 2002;99:1870–5.
25. Andrieu-Abadie N, Jaffrezou JP, Hatem S *et al*. L-Carnitine prevents doxorubicin-induced apoptosis of cardiac myocytes: role of inhibition of ceramide generation. *Fed Am Soc Exp Biol J* 1999;13:1501–10.
26. Kumaran S, Deepak B, Naveen B *et al*. Effects of levocarnitine on mitochondrial antioxidant systems and oxidative stress in aged rats. *Drugs RD* 2003;4:141–7.
27. Lamendola C, Abbasi F, Chu JW *et al*. Comparative effects of rosuvastatin and gemfibrozil on glucose, insulin, and lipid metabolism in insulin-resistant, nondiabetic patients with combined dyslipidemia. *Am J Cardiol* 2005;95:189–93.
28. Güçlü F, Ozmen B, Hekimsoy Z *et al*. Effects of a statin group drug, pravastatin, on the insulin resistance in patients with metabolic syndrome. *Biomed Pharmacother* 2004;58:614–8.
29. Mingrone G, Greco AV, Capristo E *et al*. L-carnitine improves glucose disposal in type 2 diabetic patients. *J Am Coll Nutr* 1999;18:77–82.
30. Proulx F, Lacroix J, Qureshi IA *et al*. Acquired carnitine abnormalities in critically ill children. *Eur J Pediatr* 1997;156:864–9.
31. Power RA, Hulver MW, Zhang JY *et al*. Carnitine revisited: potential use as adjunctive treatment in diabetes. *Diabetologia* 2007;50:824–32.
32. Poorabbas A, Fallah F, Bagdadchi J *et al*. Determination of free L-carnitine levels in type II diabetic women with and without complications. *Eur J Clin Nutr* 2007;61:892–5.
33. Mamoulakis D, Galanakis E, Dionyssopoulou E *et al*. Carnitine deficiency in children and adolescents with type 1 diabetes. *J Diabetes Complicat* 2004;18:271–4.
34. Ido Y, McHowat J, Chang KC *et al*. Neural dysfunction and metabolic imbalances in diabetic rats. Prevention by acetyl-L-carnitine. *Diabetes* 1994;43:1469–77.
35. Nakamura J, Koh N, Sakakibara F *et al*. Polyol pathway hyperactivity is closely related to carnitine deficiency in the pathogenesis of diabetic neuropathy of streptozotocin-diabetic rats. *J Pharmacol Exp Ther* 1998;287:897–902.
36. Teoman Uysal K, Wiesbrock SM, Marino MW *et al*. Protection from obesity-induced insulin resistance in mice lacking TNF- α function. *Nature* 1997;389:610–4.
37. Haque M, Sanyal AJ. The metabolic abnormalities associated with non-alcoholic fatty liver disease. *Best Pract Res Clin Gastroenterol* 2002;16:709–31.
38. Furukawa H, Carrol R, Swift H *et al*. Long-term elevation of free fatty acids leads to delayed processing of proinsulin and prohormone convertases 2 and 3 in the pancreatic beta-cell line MIN6. *Diabetes* 1999;48:1395–401.
39. Oakes ND, Camilleri S, Furler SM *et al*. The insulin sensitizer, BRL 49653, reduces systemic fatty acid supply and utilization and tissue lipid availability in the rat. *Metabolism* 1997;46:935–42.
40. Seccombe DW, James L, Hahn P *et al*. L-carnitine treatment in the hyperlipidemic rabbit. *Metabolism* 1987;36:1192–6.
41. Liang LJ, Yin XY, Luo SM *et al*. Study of the ameliorating effects of carnitine on hepatic steatosis induced total parenteral nutrition in rats. *World J Gastroenterol* 1999;5:312–5.
42. Fabbri E, Mohammed BS, Magkos F *et al*. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* 2008;134:424–31.
43. Diraison F, Moulin P, Beylot M. Contribution of hepatic *de novo* lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab* 2003;29:478–85.
44. Bowyer BA, Miles JM, Haymond MW *et al*. L-carnitine therapy in home parenteral nutrition patients with abnormal liver tests and low plasma carnitine concentration. *Gastroenterology* 1988;94:434–8.
45. Bowyer BA, Fleming CR, Haymond MW *et al*. L-carnitine: effect of intravenous administration on fuel homeostasis in normal subjects and homeparenteral-nutrition patients with low plasma carnitine concentrations. *Am J Clin Nutr* 1989;49:618–23.
46. Ahern DA, Mitchell ME. Liver function in protein-energy malnutrition measured by cinnamic acid tolerance and benzoic acid tolerance: effect of carnitine supplementation. *Br J Nutr* 1989;61:209–21.
47. Cassader M, Gambino R, Musso G *et al*. Postprandial triglyceride-rich lipoprotein metabolism and insulin sensitivity in nonalcoholic steatohepatitis patients. *Lipids* 2001;36:1117–2419.