

Hepatoprotective effect of combination of L-carnitine and magnesium-hydroxide in nonalcoholic fatty liver disease patients: a double-blinded randomized controlled pilot study

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Abstract. – OBJECTIVE: Non-alcoholic fatty liver disease (NAFLD) still has no accepted pharmacological therapy. Even though monotherapy of L-carnitine or magnesium supplementation exhibits an essential beneficial role in NAFLD treatment, and despite that new NAFLD treatment strategies focus on combination therapies, the combination of L-carnitine with magnesium has not yet been examined in NAFLD patients. We aimed to assess the efficacy of L-carnitine in combination with magnesium in NAFLD patients.

PATIENTS AND METHODS: Double-blinded, randomized controlled trial with 22 NAFLD participants who were randomized to either control group receiving placebo for the first 8 weeks and an additional 8 weeks with CIRRHOS product (2 gr L-carnitine and 150 mg magnesium) or treatment group receiving CIRRHOS product for 16 weeks. Weight, serum aspartate aminotransferase (AST), alanine transaminase (ALT) and C-reactive protein (CRP) levels were measured monthly. Lipid profile and serum insulin levels were monitored at baseline and at week 16 of treatment. Shear-wave elastography was used to evaluate liver stiffness (LS).

RESULTS: While AST and ALT levels decreased progressively over 16 weeks of treatment in the treatment group, AST and ALT levels of the control group were increased modestly or unaffected. AST and ALT levels of the treatment group decreased by 25% ($p=0.9$) and 20% ($p=0.1$) respectively, compared to AST and ALT levels at baseline. However, serum CRP levels, insulin levels, lipid profile and LS were not affected by treatment.

CONCLUSIONS: Our findings suggest that L-carnitine with magnesium supplementation could be a potential therapy for NAFLD. However, further studies with a larger population and high-sensitivity diagnostic parameters for early stages of NAFLD are needed to elucidate L-carnitine and magnesium efficacy in NAFLD.

Key Words:

Non-alcoholic fatty liver disease, L-carnitine, Magnesium-hydroxide.

Abbreviations

ALT: alanine transaminase, AST: aspartate aminotransferase, BMI: body mass index, CRP: C-reactive protein, HDL-C: high-density lipoprotein cholesterol, kPa: kilopascals, LDL-C: low density lipoprotein cholesterol, LS: liver stiffness, NAFLD: Non-alcoholic fatty liver disease, TG: triglycerides.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is predominant worldwide and is the most common chronic liver disease in Western countries. The prevalence of NAFLD is an important public health concern. About 25% of adults worldwide have fatty livers and more than a quarter of adults with NAFLD are estimated to have nonalcoholic steatosis (NASH), a severe form of NAFLD that is characterized by hepatocellular injury and inflammation, with or without fibrosis¹.

The increasing prevalence of NAFLD is associated with non-modifiable risk factors such as age, gender, race, and genetic predisposition, and with modifiable risk factors such as obesity, dyslipidemia, type 2-diabetes and metabolic syndrome. Targeting the modifiable risk factors is an important approach for delaying or preventing NAFLD. Such interventions include lifestyle changes and nutritional components, such as L-carnitine and magnesium, which were robustly found to be associated with decreased risk of NAFLD² but these results were conflicting. The present meta-analysis was conducted to assess the association between food groups and the likelihood of NAFLD. Published literature was retrieved and screened from MEDLINE, Embase and Web of Science. Out of 7892 retrieved articles, twenty-four observational studies (fifteen cross-sectional studies and nine case-control studies).

Carnitine is a conditionally essential nutrient that is widely distributed in food from animal sources. In humans, carnitine is also obtained by endogenous biosynthesis, which predominantly occurs in the liver. Because the liver is one of the main sites for carnitine synthesis, liver disease can impair carnitine synthesis, which further exacerbates NAFLD progression. Numerous population studies have shown that L-carnitine supplementation can reduce serum levels of liver enzymes, decrease the severity of NAFLD and improve both insulin resistance and the lipid profile³⁻⁶ lipid profile, and improve liver function. The aim of the study was to assess serum chemerin in obese children with suspected NAFLD, the effect of LC on NAFLD grade, chemerin and metabolic profile. Methods Fifty obese children were compared to 50 controls. All were subjected to anthropometric assessment, liver function, fasting lipid profile, glucose/insulin (G/I). In addition, several meta-analyses have shown that L-carnitine supplementation reduces histological steatosis and non-alcoholic steatosis scores in patients with NASH⁷ the latter being confined to NASH and demanding specific treatment. We assessed the efficacy of proposed treatments for NAFLD/NASH by reviewing reports of randomized controlled trials (RCTs). Recently, Li and Zhao⁸ concluded that L-carnitine may have therapeutic effects on liver diseases, including NASH. Several experimental studies have been conducted to delineate a possible mechanism underlying the beneficial effects of L-carnitine supplementation in NAFLD. *In vitro* assay showed that L-carnitine attenuates

fructose-mediated lipid accumulation through AMPK activation, and counteracts mitochondrial damage and reactive oxygen species production⁹. Early work by Mollica et al¹⁰ showed that in a mouse model of a methionine-choline deficient diet, which is a well-established dietary model of NAFLD, L-carnitine supplementation decreased hepatic lipid accumulation and oxidative stress, and reduced hepatic fibrosis.

Similar to L-carnitine, magnesium has also been suggested to play a beneficial role in the management of NAFLD. Patients with NAFLD may have low serum levels of magnesium, which are associated with impaired absorption and transportation due to intestinal edema and decreased albumin levels¹¹. Several epidemiological studies have demonstrated that magnesium intake is associated with a decreased risk of mortality due to liver disease. Moreover, evidence from a meta-analysis of randomized double-blinded controlled trials suggests that magnesium supplementation may attenuate risk factors for NAFLD, such as insulin resistance, high glucose levels and decreased high-density lipoprotein cholesterol (HDL-C) levels in individuals with type-2 diabetes mellitus, especially in those with low serum magnesium levels¹². Results of a recent *in vitro* study supported the positive regulatory effect of magnesium on the metabolic disorders that occur in hepatocytes under lipotoxicity. In these studies, magnesium-isoglycyrrhizinate treatment of the hepatic L02 cell line and HepaRG cells reduced the total lipid content and lipid saturation, inhibited glyceride and glycerophospholipid metabolism, downregulated the expression of metabolic enzymes involved in triglyceride biosynthesis and suppressed the unfolded protein response *via* inhibition of NF- κ B^{13,14}.

Recently, new NAFLD treatment strategies have focused on combination therapy. Evidence from clinical trials has shown that monotherapy is not sufficiently efficacious for NAFLD treatment. Thus, combining pharmacological or non-pharmacological therapy may increase treatment efficacy. For example, a combination of antidiabetic drugs with anti-NASH drugs may improve both liver and diabetic effects¹⁵.

Although previous studies have demonstrated that a monotherapy of L-carnitine or magnesium exhibits an essential, beneficial role in NAFLD treatment, the combination of L-carnitine with magnesium has not yet been examined in NAFLD patients. Therefore, in this double-blinded,

randomized controlled pilot study, we aimed to assess the efficacy of L-carnitine in combination with magnesium in NAFLD treatment.

Patients and Methods

Patients

Eligible patients from 18 to 75 years of age with a clinical diagnosis of NAFLD on the basis of liver biopsy or liver imaging (shear wave elastography) were involved in the study. The inclusion criteria were as follows: (1) Patients diagnosed with NAFLD after eliminating other fatty liver etiology; (2) Patients who read and understood the informed consent form and gave their written consent to participate in the study. The exclusion criteria were as follows: (1) Patients with liver cirrhosis; (2) Patients with liver failure; (3) Patients with chronic or acute renal failure (a creatinine clearance test calculated as less than 50 ml/min or blood creatinine above 1.5 mg/dl); (4) Patients with heart failure or New York Heart Association grade 3-4; (5) Patients with active cancer; (6) Patients with fatty liver due to non-NAFLD reasons (such as patients consuming a high alcohol level – over 10 gr per day in women and over 20 gr per day in men); (7) Patients who consume drugs from the estrogen family, such as methotrexate and chloroquine; (8) Patients with a history of Cushing’s disease; (9) Patients receiving total parenteral nutrition for the past six months; (10) Patients with chronic liver disease, such as α 1-antitrypsin, hemochromatosis, Wilson, toxic injury, autoimmune; (11) Patients receiving acid treatment; (12) Patients receiving coumadin therapy; (13) Special populations such as pregnant women and incapacitates.

Study Design

The study was designed by the investigators and was conducted according to Good Clinical Practice guidelines, the Helsinki Declaration and all applicable regulations, with independent Ethics Committee and Institutional Review Board approval.

From November 2018 to February 2019, 22 eligible study participants who were diagnosed with NAFLD according to liver biopsy or liver imaging were invited to a university-affiliated medical center and recruited to the study after signing an informed consent form. Study participants were double-blind randomized to two groups. Group 1 (control group) received placebo (mineral water) for the first 8 weeks and an additional 8 weeks with the “CIRRHOS” product, which contains L-carnitine and magnesium (daily dose of 15 ml). Group 2 (treatment group) received the CIRRHOS product for 16 weeks (daily dose of 15 ml). “CIRRHOS” (Tudu Holdings LTD, Tel-Aviv, Israel) contains 2 gr of amino acid L-carnitine, 150 mg of magnesium-hydroxide and 10 mg vitamin C. The experimental design of the study is illustrated in Figure 1.

Sealed kits, numbered from one to twenty-two, were assigned to study participants in chronological order upon recruitment. Each kit contained 4 numbered bottles: each bottle sufficing for 4 weeks. The study group kit contained 4 bottles of CIRRHOS product. The placebo group kit contained 2 bottles of mineral water and 2 remaining bottles of CIRRHOS product. The order of the bottles in the kits of both groups was randomized by the provider, and the randomization key was undisclosed until the end of the study.

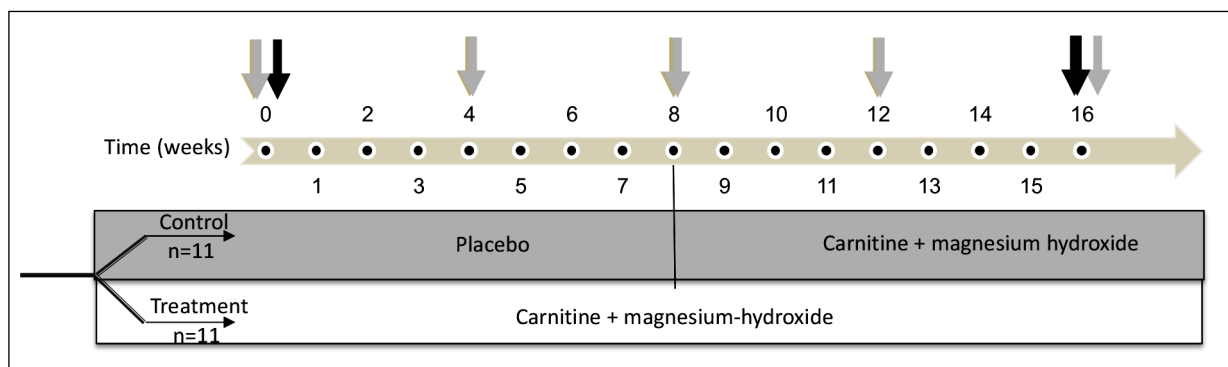


Figure 1. The experimental design of the study. Gray arrows indicate evaluation of body weight, serum transaminases levels, C-reactive protein (CRP). Black arrows indicate evaluation of serum insulin levels, lipid profile and shear wave elastography measurements for assessment of liver fibrosis and steatosis.

Clinical Evaluation

Study participants were monitored over the phone on a weekly basis and during visits on a monthly basis. Visits included collection of various parameters such as body weight, serum transaminases levels and CRP. Lipid profile [total-cholesterol, low density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides (TG)] and serum insulin levels were monitored at baseline and at week 16 of treatment. These parameters were measured with standard clinical chemistry laboratory methods.

Shear Wave Elastography

LS (kilopascals (kPa)) was performed using shear wave elastography (SuperSonic Imagine, Aix-en-Provence, France). Shear wave elastography was conducted at baseline and at the end of week 16 to compare the degree of liver fibrosis before and after treatment. The ranking of liver scarring was calculated according to the METAVIR fibrosis score, from F0 to F4, with an accuracy between 86% and 98% and specificity between 90% and 93%. Liver fibrosis was classified into five stages: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal 2 disease markers fibrosis with rare septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.

Shear wave elastography was performed by a trained and experienced sonographer. All patients fasted for 8 hours prior to the examination and were instructed to stop drinking alcohol and exercising for 12 hours and 20 min respectively, prior to the examination. Measurements were performed in a supine position with the right arm elevated. Scans were obtained after a small breath hold, using an intercostal approach, with sampling greater than 1 cm from the liver surface and away from major vessels. At least 2 measurements were obtained from the right lobe of the liver. Images were interpreted by a single, blinded, senior radiologist.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows v.21.0 (IBM, Armonk, NY, USA) and GraphPad software package 6 (La Jolla, CA, USA). Categorical variables are presented as means and standard deviations. The difference in the continuous data between the two groups was examined using Student *t*-tests or the Wilcoxon test where appropriate. For categorical variables, we used

the chi-square test. Paired *t*-tests were used to assess differences between baseline and 4 months within each group. Statistical significance was considered to be $p < 0.05$. Sample size was determined according to previously described methods for pilot studies¹⁶⁻¹⁹, and according to the time frame and budget for the study.

Results

Baseline Characteristics

The subjects included 22 patients with NAFLD. Of the 22 participants, 11 control patients received placebo (mineral water) for the first 8 weeks and CIRRHOS product for an additional 8 weeks, and 11 treatment patients received a CIRRHOS product containing 2 gr L-carnitine and 150 mg magnesium for 16 weeks. Statistically significant gender differences were found between the control (8 female, 3 male) and treatment group (2 female, 9 male) ($p=0.01$), with a greater number of female patients in the control group compared to the treatment group. Age of patients in the control group (53.8 ± 4.2 yrs.) was significantly lower than the treatment group (40.4 ± 3.3 yrs.) ($p=0.02$). No significant differences were observed between control and treatment group in AST ($p=0.69$), ALT ($p=0.9$) and CRP ($p=0.99$) in plasma at baseline. Also, we did not observe significant differences between the control and treatment groups in baseline levels of total cholesterol ($p=0.07$), TG ($p=0.36$), LDL-C ($p=0.06$), HDL-C ($p=0.47$), and insulin ($p=0.14$). Baseline shear wave elastography results showed no significant differences at LS between control (8.1 ± 1.2 kPa) and treatment group (6.2 ± 0.3 kPa), ($p=0.16$). The participants' characteristics are shown in Table I.

Weight Stability in Both Control and Treatment Groups

Body weight of both control and treatment was constant during 16 weeks of the study. The mean baseline body weight of control was 94.5 ± 2.7 kg compared to 94.2 ± 3.2 kg at week 16 of the treatment ($p=0.9$). Similarly, in the treatment group, the mean baseline body weight was 90.8 ± 6.2 kg compared to 89.7 ± 6.2 kg at week 16 of the treatment ($p=0.9$). Also, body weight of control was not significantly different from the treatment

Table I. Participants' demographics and characteristics.

Parameter	Control group (n = 11)	Treatment group (n = 11)	p-value
Demographic parameters			
Gender, female	8 (72.8%)	2 (18.2%)*	0.01
Age (years)	53.8 ± 4.2	40.4 ± 3.3*	0.02
Weight (Kg)	94.5 ± 2.7	90.9 ± 6.2	0.62
BMI (Kg/m ²)	35.0 ± 1.1	31.2 ± 1.5	0.07
Liver function			
AST (U/L)	40.6 ± 6.0	52.0 ± 6.6	0.69
ALT (U/L)	73.6 ± 17.5	76.1 ± 6.8	0.9
CRP (mg/L)	8.78 ± 1.9	8.73 ± 2.8	0.99
Lipid profile			
Cholesterol (mg/dL)	144.8 ± 7.9	175.1 ± 13.9	0.07
Triglycerides (mg/dL)	137.4 ± 14.2	162.5 ± 23.2	0.36
LDL-C (mg/dL)	75.6 ± 7.2	103.1 ± 11.9	0.06
HDL-C (mg/dL)	41.7 ± 2.8	38.7 ± 2.9	0.47
Insulin level (μIU/mL)	28.5 ± 6.3	16.7 ± 2.8	0.14
Liver stiffness			
Fibroscan (METAVIR fibrosis score)	8.1 ± 1.2	6.2 ± 0.3	0.16

Data are shown as No. (%) or mean ± SEM. * $p < 0.05$; mean values are statistically significantly different. Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Body Mass Index (BMI), C-Reactive Protein (CRP), High-Density Lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C).

group at baseline ($p=0.62$) and at week 16 of the treatment ($p=0.56$) (Figure 2A).

In control, the mean BMI at baseline was 35 ± 1.1 kg/m², compared to 34.9 ± 1.3 kg/m² at week 16 of the treatment ($p=0.95$). Mean BMI of the treatment group was 31.2 ± 1.5 kg/m² at baseline, compared to 30.7 ± 1.4 kg/m² at week 16 of the treatment ($p=0.8$). Mean BMI of the control group was not significantly different from the mean BMI of the treatment group at baseline ($p=0.07$) and at week 16 of the treatment ($p=0.05$) (Figure 2B).

Serum Transaminases Levels Decreased Progressively in Treatment Group

In order to assess the effect of L-carnitine with magnesium supplementation on liver function, we examined serum AST and ATL enzymes. As expected, in the treatment group, serum AST levels decreased by 25% at week 16 compared to baseline, but this did not reach statistical significance ($p=0.08$). Unlike the treatment group, in the control group, serum AST levels insignificantly increased by 21% at week 16 compared to baseline ($p=0.8$). At baseline, serum AST levels

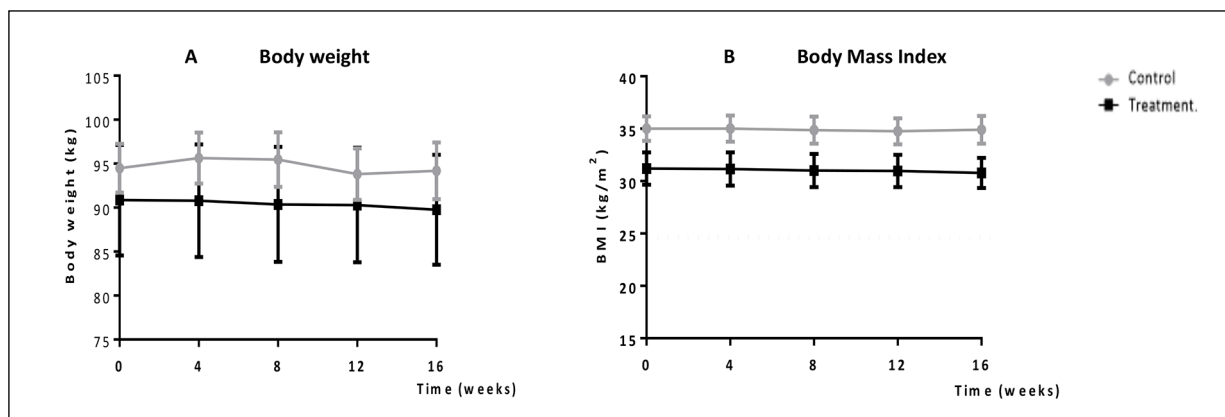


Figure 2. Longitudinal measurements of A) body weight and B) body mass index (BMI) of control and treatment groups over 16 weeks of study. Results presented as mean ± SEM. Gray dashed line indicates the upper limit of the normal range of each value. n=10-11/group.

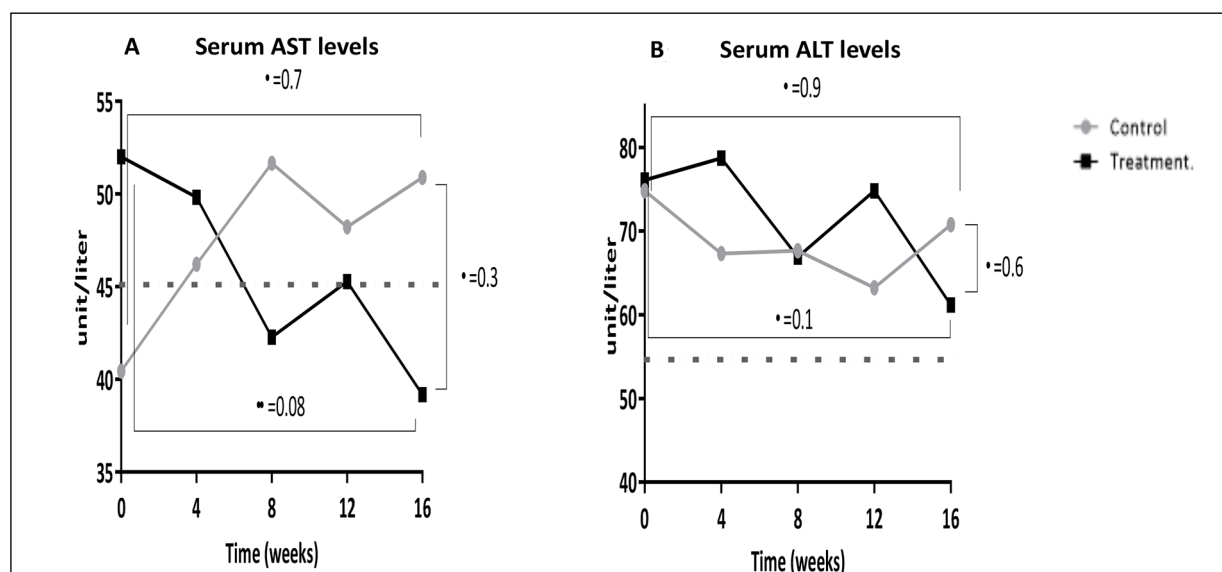


Figure 3. Mean serum liver transaminases over 16 weeks of study in control and treatment group. **A**, Serum aspartate aminotransferase (AST) U/L. **B**, Alanine transaminase (ALT) U/L. Gray dashed line indicates the upper limit of the normal range of each value. The difference between the two groups was examined using Student's *t*-tests to assess differences between baseline and 4 months within each group. $n=10-11$ /group.

of the control group (40.6 ± 6.0 U/L) were not significantly different from AST levels of the treatment group (52 ± 6.6 U/L) ($p=0.7$). However, at week 16 of the treatment, serum AST levels of the treatment group (39.1 ± 2.6 U/L) were 23% lower than control (50.9 ± 11.7 U/L), but this did not reach statistical significance ($p=0.3$) (Figure 3A).

Similarly, serum ALT levels of treatment group at week 16 of the treatment decreased by 20% compared to ALT levels at baseline, but this did not reach statistical significance ($p=0.1$). However, in the control group, serum ALT levels at week 16 of the treatment decreased only by 5% compared to ALT levels at baseline ($p=0.9$). At baseline, serum ALT levels of control (73.6 ± 17.5 U/L) were not significantly different from serum ALT levels of the treatment group (76 ± 6.8 U/L) ($p=0.9$). However, at week 16 of the treatment, serum ALT levels of the treatment group (61.1 ± 6.3 U/L) were 15% lower than control (70.7 ± 19.3 U/L) ($p=0.6$) (Figure 3B). We also examined serum CRP levels. CRP is an acute-phase reactive protein that is predominantly produced in the liver and is a marker of systemic inflammation and tissue injury²⁰. Serum CRP levels were decreased in both control and treatment groups. Serum CRP levels of the control group at week 16 of the treatment decreased by 37% compared to baseline levels ($p=0.2$). In the treatment group, serum CRP levels decreased at week 16 of treatment by 19% compared to baseline levels ($p=0.6$). We did

not observe significant differences in serum CRP levels between the control and treatment groups at baseline or at week 16 of treatment ($p=0.9$, $p=0.6$ respectively) (Figure 4).

Since NAFLD is associated with an imbalanced metabolic network that involves glucose and lipid metabolism, we examined plasma insulin levels and lipid profile, which are consis-

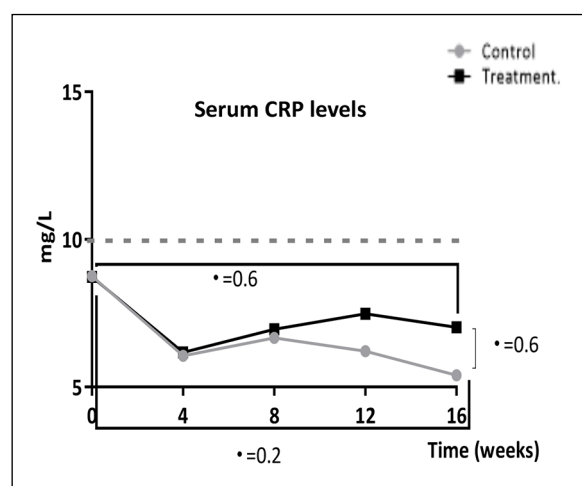


Figure 4. Mean serum liver C-reactive protein (CRP) (mg/L) over 16 weeks of study in control. Gray dashed line indicates the upper limit of the normal range of each value. The difference between the two groups was examined using Student *t*-tests to assess differences between baseline and 4 months within each group. $n=10-11$ /group.

tent with NAFLD development^{21,22} an infusion technique is devised that provides a more specific measure of cellular resistance to insulin mediated glucose uptake. This technique is used to study the relationship between fasting plasma insulin level and resistance to insulin mediated glucose uptake in twenty two patients with normal oral glucose tolerance, fourteen patients with impaired glucose tolerance, and fourteen patients classified as having chemical diabetes mellitus. The results indicate a highly significant positive correlation between the degree of insulin resistance and fasting plasma insulin concentration ($r = .69, p < .0001$). In the control group, baseline plasma insulin levels ($26.7 \pm 5.9 \mu\text{IU/mL}$) were not significantly different from insulin levels at week 16 of treatment ($23.9 \pm 5.6 \mu\text{IU/mL}$) ($p=0.7$). Similarly, in the treatment group, baseline plasma insulin levels ($16.7 \pm 2.8 \mu\text{IU/mL}$) were not significantly different from

insulin levels at week 16 of treatment ($16.3 \pm 1.9 \mu\text{IU/mL}$) ($p=0.9$). We did not observe significant differences in serum insulin levels between the control and treatment groups at baseline (28.5 ± 6.3 vs. $16.7 \pm 2.8 \mu\text{IU/mL}$, $p=0.09$) or at week 16 of treatment (23.9 ± 5.6 vs. $16.3 \pm 1.9 \mu\text{IU/mL}$, $p=0.2$) (Figure 5A).

In control, baseline plasma cholesterol levels were not significantly different from cholesterol levels at week 16 of treatment (144.8 ± 7.9 vs. $157 \pm 8.9 \text{ mg/dl}$, $p=0.3$). Likewise, in the treatment group, baseline plasma cholesterol levels were not significantly different from cholesterol levels at week 16 of treatment (175.1 ± 13.9 vs. $173.3 \pm 13.4 \text{ mg/ml}$, $p=0.9$). There were no significant differences between the control and treatment groups in cholesterol levels at baseline (144.8 ± 7.9 vs. $175.1 \pm 13.9 \text{ mg/ml}$, $p=0.07$) and at week 16 (157 ± 8.9 vs. $173.3 \pm 13.4 \text{ mg/ml}$, $p=0.3$) (Figure 5B).

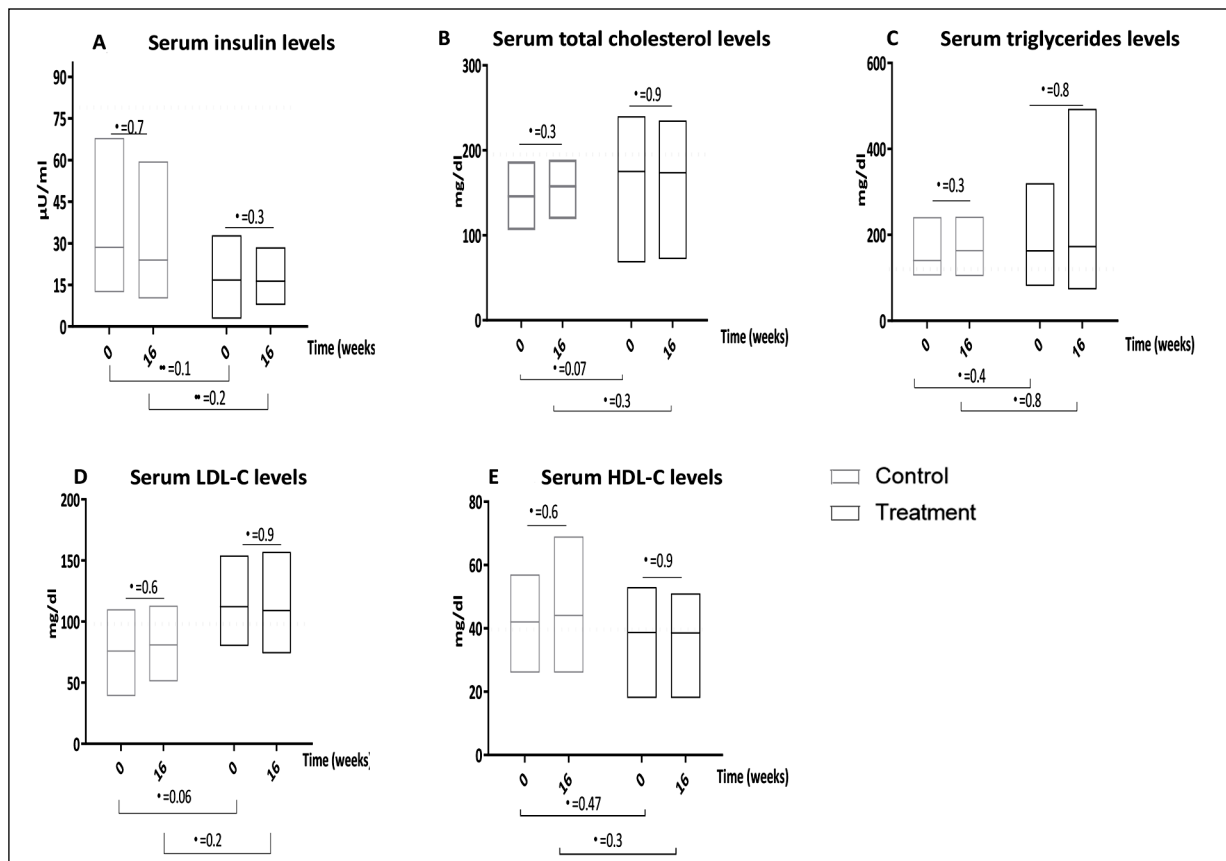


Figure 5. Mean serum levels of insulin and lipid profile at baseline and at week 16 of treatment in control and treatment group. **A,** Serum insulin $\mu\text{U/L}$. **B,** Serum total cholesterol mg/dl. **C,** Serum triglycerides mg/dl. **D,** Serum low-density lipoprotein cholesterol (LDL-C) mg/dl. **E,** Serum high-density lipoprotein cholesterol (HDL-C) mg/dl. Gray dashed line indicates the upper limit of the normal range of each value. The difference between the two groups was examined using Student *t*-tests to assess differences between baseline and 4 months within each group. $n=9-11/\text{group}$.

Moreover, in the control group, baseline plasma TG levels (137.4 ± 14.2 mg/ml) were not significantly different from TG levels at week 16 of treatment (163.2 ± 16.2 mg/ml) ($p=0.3$). In the treatment group, we also did not observe significant differences between baseline plasma TG levels (162.5 ± 23.2 mg/ml) and TG levels at week 16 of treatment (172.5 ± 35.8 mg/ml, $p=0.8$). We did not observe significant differences in TG levels between the control and treatment groups at baseline (137.4 ± 14.2 vs. 162.5 ± 23.2 mg/ml, $p=0.3$) or at week 16 of treatment (163.2 ± 16.2 vs. 172.5 ± 35.8 mg/ml, $p=0.8$) (Figure 5C).

In the control group, plasma LDL-C levels at baseline (75.6 ± 7.2 mg/ml) were not statistically different from LDL-C levels at week 16 of treatment (80.8 ± 7.7 mg/ml) ($p=0.6$). In the treatment group, baseline plasma LDL-C levels (103.1 ± 11.9 mg/ml) were not significantly different from LDL-C levels at week 16 of treatment (101.2 ± 11.8 mg/ml) ($p=0.9$). Further, baseline plasma LDL-C levels of the control group (75.6 ± 7.2 mg/ml) were not significantly different from baseline LDL-C levels of the treatment group (103 ± 11.9 mg/ml) ($p=0.06$). Likewise, at week 16 of treatment, plasma LDL-C levels of control (80.8 ± 7.7 mg/ml) were not significantly different from LDL-C levels of treatment group (101.2 ± 11.8 mg/ml) ($p=0.2$) (Figure 5D).

Plasma HDL-C levels of control at baseline (41.7 ± 2.8 mg/ml) were not significantly different from HDL-C levels at week 16 of treatment (44.1 ± 3.8 mg/ml) ($p=0.6$). In the treatment group, plasma HDL-C levels at baseline (38.7 ± 2.9 mg/ml) were not significantly different from HDL-C levels at week 16 of treatment (38.5 ± 2.9 mg/ml) ($p=0.9$). HDL-C levels of the control group were not significantly different from HDL-C levels of the treatment group both at baseline ($p=0.47$) and at week 16 of treatment ($p=0.3$) (Figure 5E).

Liver Stiffness Scores Were Not Affected by L-carnitine with Magnesium Treatment

Next, we measured LS by shear wave elastography to determine the effect of L-carnitine with magnesium supplementation on LS and the change in liver disease progression. In the control group, mean LS scores at baseline (8.1 ± 1.2 kPa) were not different from LS scores at week 16 of treatment (8.1 ± 1.6 kPa) ($p=0.98$). Likewise, in the treatment group, LS scores at baseline (6.2 ± 0.3 kPa) were not different from LS scores at week 16 of treatment (6.3 ± 0.4 kPa) ($p=0.7$). At

baseline, the mean LS scores of the control group (8.1 ± 1.2 kPa) were not significantly different from LS scores of the treatment group (6.1 ± 0.3 kPa) ($p=0.1$). Similarly, at week 16 of treatment there were no differences in LS scores between the control (8.1 ± 1.6 kPa) and treatment groups (6.3 ± 0.4 kPa) ($p=0.2$) (Figure 6).

Discussion

Even though both L-carnitine and magnesium may play a key role in maintaining liver function through their effect on lipid metabolism, the combined effect of L-carnitine with magnesium was not previously studied in NAFLD patients. We, therefore, sought to determine the effect of L-carnitine with magnesium supplementation on liver function in NAFLD patients. Numerous studies have shown that L-carnitine supplementation reduces steatosis and is advantageous for the treatment of NAFLD. Also, low serum magnesium concentration has been shown to be associated with NAFLD¹². Therefore, we predicted that a combination of L-carnitine with magnesium will improve liver function in NAFLD patients. Interestingly, our prediction was only partially upheld. Although our study con-

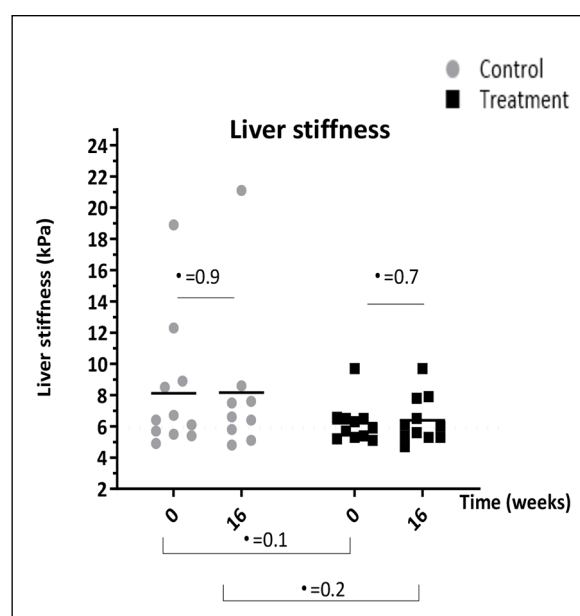


Figure 6. Mean liver stiffness (kPa) at baseline and at week 16 of treatment in control and treatment group. Gray dashed line indicates the upper limit of the normal liver stiffness. The difference between the two groups was examined using Student *t*-tests. $n=10-11$ /group.

firmed a progressive decrease of AST and ALT enzymes in NAFLD patients supplemented with L-carnitine and magnesium, metabolic parameters such as serum insulin levels and serum lipid profile were not affected. Unexpectedly, LS was not affected by L-carnitine with magnesium supplementation.

Since obesity is an independent risk factor for NAFLD¹³, we expected that L-carnitine with magnesium supplementation may improve BMI. However, our results showed that while the body weight and BMI of both control and treatment groups indicated obesity (BMI>30), both measures were not affected by L-carnitine with magnesium supplementation. Contrary to our findings, a recent meta-analysis demonstrated that L-carnitine supplementation decreased weight and BMI²³. Also, the 30-year longitudinal CARDIA study, which included about 5000 subjects, indicated that magnesium intake is inversely associated with the incidence of obesity²⁴. In addition, in animal models of diet-induced obesity, magnesium supplementation prevents the accumulation of adipose tissue²⁵. The contrasting results can be explained by several differences between the design of these studies and our study, such as the dosage of L-carnitine and magnesium, duration of treatment, and the health status and life-habits background of the participants.

As expected, L-carnitine and magnesium supplementation resulted in a sharp decline of serum AST levels compared to the slow reduction of serum ALT levels. These results can be explained by the fact that the half-life of the ALT enzyme is longer than the AST enzyme (47 hrs. vs. 17 hrs., respectively)²⁶. As predicted, in our study we observed a progressive reduction of circulating ALT and AST levels caused by L-carnitine with magnesium supplementation. This is consistent with the findings of Pirmadah et al²⁷ previous findings were equivocal. The current systematic review and meta-analysis of randomized controlled clinical trials (RCTs), who concluded that L-carnitine supplementation significantly improves circulating ALT and AST levels. Experimental studies have also shown that magnesium-supplemented rats present decreased AST and ALT levels²⁸.

The insignificant decrease of AST and ALT levels that was observed in our study may be explained by the relative low dosage of carnitine (2000 mg/day). Indeed, previous studies showed that L-carnitine is more effective in reducing transaminases with intervention doses of more

than 2000 mg/day. Another explanation for the insignificant changes in AST and ALT levels may lie in the type of magnesium salt; in our study, we used magnesium-hydroxide, while studies that showed a significant effect of magnesium on transaminases used other magnesium salts, such as isoglycyrrhizinate²⁹.

We observed that serum CRP levels were not affected in the control and treatment groups. Previous studies showed inconsistent results regarding the correlation between circulating CRP and NAFLD. Indeed, elevated CRP levels were observed in NASH and not in simple steatosis cases. Consistent with this, in our study the participants suffered from mild-moderate NAFLD, which may explain the fact that circulating CRP levels were unaffected³⁰.

Further, we examined features of insulin resistance, which is the pathophysiological hallmark of NAFLD, including serum fasting insulin and lipid profile. However, abnormalities in serum insulin levels and lipid components (total cholesterol, HDL-C, LDL-C, and TG) at baseline and after L-carnitine with magnesium supplementation were not observed. The unaffected serum insulin and lipid profile could be due to early stages of NAFLD in our participants, which are characterized by steatosis without abnormal metabolic features. Indeed, our shear wave elastography results of LS also showed a mild-moderate fibrosis (<8kPa) for both control and treatment group at baseline and after treatment. Previous studies indicated that increasing grades of NAFLD are associated with abnormal features of insulin resistance such as lipid profile and serum insulin levels³¹. There are several limitations to this study: (1) The main limitation of this study is the small sample size. However, although the study's sample size is small because of a limited patient-pool size, a double-blind, placebo-controlled design was used to counterbalance this problem. (2) In our study we used ultrasonography to assess NAFLD severity, although liver biopsy is the gold standard for diagnosis of NAFLD. This is because liver biopsy is an invasive and difficult procedure for the patients, associated with sampling mistakes and not free of risks, and thus not practical and arguably unethical in asymptomatic cases. (3) At the clinical level, the bioavailability and dosage of the different magnesium salts deserves clarification. In our study, we used magnesium-hydroxide, which is used as a simple magnesium supplement and has a relatively high bioavailability without noticeable

side effects³². (4) Another important limitation lies in the fact that the control group in our study received a placebo for the first 8 weeks, and then received the CIRRHOS product containing L-carnitine and magnesium for an additional 8 weeks. The possibility of receiving a supplement enhanced participant recruitment and retention in the study.

Conclusions

In the present study we showed that L-carnitine with magnesium supplementation could be a potential, safe tool for NAFLD treatment. Further studies with a larger population should examine L-carnitine with magnesium efficacy in early and advanced NAFLD stages using parameters with high sensitivity and specificity to differentiate simple steatosis from steatohepatitis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The study was performed according to the guidelines of the Helsinki Declaration on Human Experimentation and was approved by the Ethical Committee of the Haemek Medical Center (IRB number 0068-16-EMC).

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Authors' Contribution

Conception and design of the study, Rawi Hazzan, Ziv Neeman; acquisition of data, Wasim Slim, Mazen Elias; analysis and interpretation of data, Nur Abu Ahmad; drafting the article or making critical revisions related to relevant intellectual content of the manuscript, Nur Abu Ahmad; supervision, Rawi Hazzan, Ziv Neeman; validation and final approval of the version of the article to be published, Rawi Hazzan, Ziv Neeman, Nur Abu Ahmad.

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Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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