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Author post-print (accepted) deposited by Coventry University's Repository

Pirmadah, F, Ramezani-Jolfaie, N, Mohammadi, M, Talenezhad, N, Clark, C & Salehi-Abargouei, A 2020, 'Does L-carnitine supplementation affect serum levels of enzymes mainly produced by liver? A systematic review and meta-analysis of randomized controlled clinical trials', *European Journal of Clinical Nutrition*, vol. 59, no. 5, pp. 1767–1783.

<https://doi.org/10.1007/s00394-019-02068-4>

DOI 10.1007/s00394-019-02068-4

ISSN 0954-3007

ESSN 1476-5640

Publisher: Springer

The final publication is available at Springer via <http://dx.doi.org/10.1007/s00394-019-02068-4>

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Does L-carnitine supplementation affect serum levels of enzymes mainly produced by liver? A systematic review and meta-analysis of controlled clinical trials

Farzaneh Pirmadah^{†1,2}, Nahid Ramezani-Jolfaie^{†1,2}, Mohammad Mohammadi^{1,2}, Nasir Talenezhad^{1,2}, Cain C. T. Clark³, Amin Salehi-Abargouei^{1,2}

[†]*These authors (FP and NRJ) contributed equally to this work.*

¹*Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

²*Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

³*Centre for Sport, Exercise and Life Sciences, Coventry University, Coventry, CV1 5FB, U.K.*

Running title: L-carnitine and liver enzymes

Corresponding Author:

Amin Salehi-Abargouei, PhD in Nutritional Sciences
Department of Nutrition
School of Public Health
Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Tel: +98-35- 31492229
Fax: +98-35-38209119
Email: abargouei@ssu.ac.ir
Alternate email: abargouei@gmail.com

Tables: 4

Figures: 3

Word count: 3627

ABSTRACT

Background & Aims: L-carnitine supplementation is proposed to be associated with reduced liver enzymes levels; however, previous findings are equivocal. The current systematic review and meta-analysis of controlled clinical trials was performed to assess the effect of L-carnitine supplementation on liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGTP)] levels.

Methods: Online databases, including PubMed, Web of science, Scopus, and Google Scholar, as well as the reference lists of identified relevant studies were searched from database inception up to June 2019. The risk of bias in individual studies was assessed using Cochrane Collaboration's tool. Data were pooled using the random-effects model and expressed as mean differences (MDs) with 95% confidence intervals (CIs).

Results: In total, nineteen trials (1206 participants) met the eligibility criteria. Intervention duration ranged from 2 to 48 weeks and L-carnitine supplementation dose ranged from 500 to 4000 mg/day. L-carnitine supplementation significantly reduced serum ALT (MD = -10.97 IU/L, 95% CI: -16.46, -5.48), AST (MD = -9.03 IU/L, 95% CI: -12.73, -5.33), and GGTP (MD = -7.88 IU/L, 95% CI: -12.11, -3.64) levels. The subgroup analysis showed that L-carnitine might be more effective in reducing liver enzymes with higher doses (≥ 2000 mg/day), longer treatment durations (> 12 weeks), and also among patients with liver diseases.

Conclusion: L-carnitine supplementation significantly improves circulating ALT, AST and GGTP levels; therefore, it might positively affect liver function, especially among patients with liver diseases.

Key words: L-carnitine; alanine aminotransferase (ALT); aspartate aminotransferase (AST); Systematic Review; Meta-Analysis

INTRODUCTION

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are also known as serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), respectively, are found in the liver, heart cells, red blood cells, muscle tissue, and other organs, such as the pancreas and kidneys; however they are mainly produced in the liver (1, 2). These enzymes play a key role in transferring the α -amino group from alanine and aspartate, to the α -keto group of ketoglutaric acid to produce pyruvic acid and oxalacetic acid respectively, which are important in gluconeogenesis and amino acid metabolism (3-5). Although measurement of serum ALT and AST levels is primarily used for the diagnosis of hepatic disease, the elevation of aminotransferase levels is not specific to liver disease. AST, which appears in skeletal muscle, the brain, the heart and red blood cells, is less specific for liver damage (6, 7). The concentration of ALT is found to be lower than AST in all cells, except in hepatic cells; therefore, its elevation is particularly related to liver disease (8-10). Gamma-glutamyl transpeptidase (GGTP) is another nonspecific marker of liver disease and also found in other organs; it is a plasma membrane enzyme that can counteract oxidative stress by increasing intracellular glutathione synthesis (11, 12). The consumption of even small amounts of alcohol increases the GGTP levels, and therefore, may be used as a biological marker for acute or chronic alcohol abuse (13, 14).

Several epidemiological associations have been reported between these enzymes, which are regarded as markers of liver dysfunction and type 2 diabetes mellitus (15-17), cardiovascular disease (1, 18-20), and mortality from vascular and non-vascular diseases (21-23). With regard to many conditions which might increase liver enzymes, assessment and treatment should be focused on identifying and eliminating harmful agents, concurrent to applying the appropriate medical therapy and non-pharmacological treatments.

Carnitine (β -hydroxy- γ -N-trimethyl aminobutyric acid), regarded as vitamin BT, is the generic phrase for a number of compounds like L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine (24, 25). L-carnitine is the active form of carnitine, which is found in the body, food and most dietary supplements (26), and is responsible for the transfer of fatty acids to mitochondria and, consequently, involved in energy production through β -oxidation of fatty acids (27, 28). L-carnitine supplementation has been shown to be beneficial in the prevention or treatment of end-stage kidney disease (29), cardiovascular disease (30), dialysis-related hypertension (31), persistent depressive disorder (32), non-alcoholic fatty liver disease (33), and sarcopenia in patients with liver cirrhosis (34). Recently, it has been proposed that L-carnitine supplementation might be effective in reducing serum liver ALT levels (35-38). Several clinical trials have evaluated the effect of L-carnitine supplementation on the levels of liver enzymes; however, the evidence remains inconclusive. Although a number of studies provide evidence that consuming L-carnitine might be effective in reducing these enzymes (35-38), others demonstrate no significant effect on serum ALT and AST levels (39-44). To the best of our knowledge, no study has yet attempted to summarize the published evidence regarding the effect of L-carnitine supplementation on serum levels of enzymes mainly produced by the liver. Therefore, the present study aimed to perform a systematic review and meta-analysis of controlled clinical trials to assess the effect of L-carnitine supplementation on serum liver enzymes (ALT, AST, and GGTP) in adults.

MATERIALS AND METHOD

The present systematic review and meta-analysis is reported based on preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines and aimed to investigate the effect of L-carnitine supplementation on circulating ALT, AST, and GGTP levels in adults. The study protocol was registered and ethically approved by the research council of Shahid Sadoughi University of Medical Sciences (registration code: IR.SSU.SPH.REC.1398.016).

Search strategy

Relevant articles were identified by searching PubMed (www.pubmed.com), Scopus (<http://www.scopus.com>), ISI Web of Science (www.webofknowledge.com), and Google Scholar (www.scholar.google.com) from the earliest available online indexing year to June 2019. No language restriction or other filters were applied when searching the literature. The following groups of medical subject headings (MeSH) and non-MeSH keywords were used: *keywords group 1*: “carnitine”, “levocarnitine”, “vitamin BT”, “bicarnesine”, “acetylcarnitine”, “hydroxyisovalerylcarnitine”, “palmitoylcarnitine”, “L-carnitine”, “propionyl-L-carnitine”, “L-carnitine L-tartrate”, “L-Carnitine-L-tartrate”, “L-carnitine Tartrate”; *keywords group 2*: “intervention”, “trial”, “randomized”, “random”, “randomly”, “placebo”, “assignment”, “clinical trial”, “RCT”, “cross-over”, “parallel”, “steatosis”, “steatoses”, “non-alcoholic fatty liver disease”, “nonalcoholic fatty liver disease”, “NAFLD”, “nonalcoholic fatty liver”, “nonalcoholic steatohepatitis”, “nonalcoholic steatohepatitides”, “alanine transaminase”, “glutamic-alanine transaminase”, “glutamic alanine”, “alanine-2-oxoglutarate aminotransferase”, “alanine 2 oxoglutarate aminotransferase”, “ALT”, “alanine aminotransferase”, “SGPT”, “glutamic-pyruvic transaminase”, “glutamic pyruvic transaminase”, “SGOT”, “glutamic-oxaloacetic transaminase”, “glutamic oxaloacetic transaminase”, “AST”, “aspartate aminotransferases”, “aspartate apoaminotransferase”,

“aspartate transaminase”, “glutamic-oxaloacetic transaminase”, “glutamic oxaloacetic transaminase”, “L-aspartate-2-oxoglutarate aminotransferase”, “L aspartate 2 oxoglutarate aminotransferase”, “glutamate-aspartate transaminase”, “glutamate aspartate transaminase”, “GGTP”, “gamma-glutamyl transpeptidase”, “ALP”, “alkaline phosphatase”; *Keywords group 3*: “mouse”, “mice”, “rats”, “in vitro”, “pig”, “rabbit”, “rooster”, “cell”, “cow”. Group 1 keywords were combined with group 2 by “AND” operator and the “NOT” Boolean was used to remove publications with keywords group 3 in their titles/abstracts. Moreover, the keywords related to anthropometric indices, glycemic and inflammatory markers, blood pressure, lipid profile, and oxidative stress were used to find relevant studies reporting liver enzymes as secondary outcomes. The details on search strategy for PubMed, Scopus, and ISI web of science are provided in the **Supplementary Table 1**.

Eligibility criteria

The population, intervention, comparison, outcome, and study types (PICOS) criteria used for the current study are shown in **Table 1**. The following inclusion criteria were considered for selecting the relevant investigations: 1) studies which were controlled clinical trial in design (randomized or non-randomized); 2) studies which assessed the effect of L-carnitine supplementation on circulating liver enzymes [e.g. ATL, AST, and other possible markers like gamma-glutamyl transpeptidase (GGTP), and alkaline phosphatase (ALP)] levels; 3) studies conducted in adults aged 18 years and more.

Trials were excluded for the following reasons: 1) they were performed in children or adolescents aged younger than 18 years; 2) studies with ≤ 1 week (wk) of intervention duration; 3) trials in which the difference between the intervention and control group was in other components in addition to L-carnitine supplementation; 4) studies in which L-carnitine was injected intravenously or intramuscularly; 5) studies which did not assess outcomes of interest;

6) studies reporting duplicate data. In the case of several publications with the same data set, publications with more complete data were selected.

The preliminary screening of titles and abstracts of all identified articles, as well as further reviewing of the full-texts of the eligible papers, were independently done by two investigators (FP and NT), and any disagreement was resolved by consulting other authors (ASA, MM, and NRJ). In addition, the reference lists of relevant original and review articles were manually scanned to identify any other potentially eligible studies.

Data extraction

The data extracting process was done by 2 independent reviewers (FP and NT). This process was approved by other investigators (ASA, MM, and NRJ). The following information was recorded from eligible studies: the last name of the first author, the year of publication, the country in which the study was implemented, the design of the study, intervention duration (weeks), the mean/range of participants' age, the number of participants, the participants' health status, the dosage of L-carnitine supplement (mg/day), the intervention carried out in the control group, and the outcome measures. To obtain the data that were not mentioned in the studies, we emailed the corresponding authors of the eligible publications.

Risk of bias assessment in individual studies

Two independent investigators (FP and NT) assessed the risk of bias in the included studies using the Cochrane risk of bias tool. Six domains were considered: (i) random sequence generation (selection bias); (ii) allocation concealment (selection bias); (iii) blinding of participants and personnel (performance bias); (iv) blinding of outcome assessment (detection bias); (v) incomplete outcome data (attrition bias); (vi) selective reporting (reporting bias). The included investigations were judged to be "low risk of bias", "high risk of bias", or "unclear

risk of bias" regarding each domain. A study was considered as a low risk of bias if it received a low risk of bias for all domains, unclear risk if it was unclear regarding the risk of bias for one or more domains and high risk of bias if it was a high risk of bias for one or more domains (45).

Assessment of the overall quality of the meta-analysis

The overall quality of the met-evidence provided by the current study was assessed using the NutriGrade scoring system, which uses a scoring system (with maximum of 10 points) to judge the quality of meta-analyses of clinical trials conducted in the field of nutrition. This tool considers the following domains: risk of bias/study quality/study limitations (3 points), precision (1 point), heterogeneity (1 point), directness (1 point), funding bias (1 point), publication bias (1 point), and study design (2 point) (46). NutriGrade suggests 4 categories for the overall quality of meta-evidence, including high (≥ 8 points), moderate (6-7.99 points), low (4-5.99 points) and very low (≤ 3.99 points).

Statistical analysis

The mean change values from baseline to follow-up and their standard deviations (SDs) for intervention and control groups/periods were extracted to calculate the mean difference and its corresponding standard error (SE), which was used as the effect size for meta-analysis. If the change values were not reported, we calculated the SD for mean change by calculating correlation coefficients ($r = 0.76$ for ALT and $r = 0.65$ for AST) from studies which already reported SDs for baseline, after intervention and change values for the intervention and the control groups (36, 38, 40, 42, 47). The correlation coefficients were used to calculate SD for change values for other studies (45). The overall mean differences (MDs) and their corresponding 95% confidence intervals (CIs) were calculated by using the random effects

model that takes the between-study heterogeneity into account (48). The Cochran Q test and I-squared statistic (I^2 is an estimate ranging from 0-100%, with lower values indicating less heterogeneity) were used to assess the heterogeneity between studies (49). The possible sources of heterogeneity were explored by using subgroup analysis based on the health status of participants (liver disease/without liver disease), type of control group (without treatment/placebo/drug), follow-up time (≤ 12 weeks / >12 weeks), and dosage of L-carnitine supplement (<2000 mg/d / ≥ 2000 mg/d). Sensitivity analysis was used to assess the robustness of the meta-analyses results by sequentially removing individual included studies. The presence of publication bias was assessed for each outcome through statistical asymmetry tests (Egger's regression asymmetry test and Begg's adjusted rank correlation test), and also by visually inspecting Begg's funnel plot. All statistical analyses were performed using STATA, version 11.2 (Stata Corp, College Station, TX) and a two-sided P-values < 0.05 were considered as statistically significant.

RESULTS

Study selection

The literature search retrieved 6321 publications, of which 1355 papers were duplicates, and 4920 papers were excluded after screening the titles and abstracts. After full-text assessment of 46 potentially relevant records, 27 studies were excluded because they did not report the outcomes of interest (n=14) (50-63); supplemented other components in addition to L-carnitine, and the difference between the two groups/periods was not only in L-carnitine (n=3) (64-66); included duplicate data from the other studies (n=7) (67-73); administered L-carnitine intramuscularly (n=1) (74); the intervention period was lower than 2 weeks (n=1) (75); conducted in participants aged younger than 18 years (n=1) (76). Finally, 19 controlled clinical trials which studied a total of 1206 subjects were selected to be included in the present systematic review and meta-analysis (35-44, 47, 77-84). The detailed steps of the study selection process are shown in **Figure 1**.

Study and participant characteristics

Twelve studies were carried out in Asian countries (37, 40-44, 77-79, 82-84) and seven studies were done in European countries (35, 36, 38, 39, 47, 80, 81), published between 1996 and 2016. All trials used a parallel design, except one study which used cross over design (80), and the intervention period in these studies ranged between 2 and 48 wks. L-carnitine was orally administered and the intervention dose ranged between 500 to 4000 mg/day. For the control groups, ten trials used placebo controls (35, 36, 39, 44, 79-84), six trials did not prescribe any treatment (37, 40-43, 77), and three trials used drugs such as interferon- α , ribavirin, and entecavir, in which participants in the intervention group also received these drugs in addition to L-carnitine (38, 47, 78). The majority of trials included either gender, with the age ranging

from 18 to 85 years. The sample size of included studies ranged from 30 to 131 participants. Nine trials were conducted among participants who had liver diseases (36-38, 43, 47, 77-79, 81). Two trials were done in hemodialysis patients (40, 41), one study included participants with hypothyroidism (44), cystic acne patients (35) and thyroid patients (80). One study was conducted in obese women (42), three trials included healthy participants (39, 82, 83) and one study included patients with suspected acute myocardial infraction (84). The characteristics of the included studies are detailed in **Table 2**.

Risk of bias assessment

A summary of the risk of bias assessment of the included studies is presented in **Table 3**. Although the description for the random generation was well-addressed in six studies (36, 38, 41, 44, 47, 78, 79, 82), others did not explain random sequence generation method, and one of the studies was not randomized (37). Two trials included information about allocation concealment (82, 85). Four studies were considered to be a low risk of bias regarding blinding of participants and personnel (36, 39, 79-85). In the majority of included trials, the blinding of outcome assessment was unclear. Incomplete outcome data were addressed in thirteen studies (36, 38-41, 44, 47, 78, 79, 81-84). All trials were categorized as low risk of bias for selective outcome reporting. Considering the six domains of the Cochrane collaborations' risk of bias assessment tool, fifteen trials were judged to have a moderate or unclear risk, and four trials had a high risk of bias.

The effect of L-carnitine on serum alanine aminotransferase (ALT) concentrations

Seventeen trials, including 1091 participants, provided data on the effect of L-carnitine supplementation on serum ALT levels (35-43, 47, 77-81, 83, 85). The pooled effect size indicated that consuming L-carnitine significantly reduced ALT concentrations (MD = -10.97

IU/L, 95% CI: -16.46, -5.48, $P < 0.001$), with high between-study heterogeneity (Q statistic = 606.33, Cochran Q test, $P < 0.001$; $I^2 = 97.4$). Although, heterogeneity was not reduced by several subgroup analyses, the results showed that serum ALT levels were reduced to a greater magnitude in patients with liver diseases (MD = -20.25 IU/L, 95% CI: -31.22, -9.28, $P < 0.001$; **Figure 2A**), compared with subjects without liver diseases (MD = -2.84 IU/L, 95% CI: -5.33, -0.34, $P = 0.026$). In another subgroup analysis, based on the type of control group, in studies which administered placebo or drug for the control group compared with studies that did not use any treatment for controls, a significant reduction in the levels of ALT was observed. There was also a significant reduction in serum ALT concentrations following L-carnitine supplementation in studies which used high doses of L-carnitine (≥ 2000 mg/day) for supplementation (MD = -14.08 IU/L, 95% CI: -22.72, -5.44, $P = 0.001$; **Figure 3A**). However, significant changes were not observed in lower intervention doses. Moreover, the reducing effect of L-carnitine on serum ALT levels was seen in both subgroups of duration; however, the effect was lower for studies with a duration of shorter than 12 weeks. **Table 4** shows the meta-analysis results for subgroup as well as overall analyses.

The effect of L-carnitine on serum aspartate aminotransferase (AST) concentrations

The results of the overall analysis of eighteen studies with 1087 participants (35-43, 47, 77, 79-85) indicated that circulating AST levels following L-carnitine supplementation was significantly decreased compared to control groups (MD = -9.03 IU/L, 95% CI: -12.73, -5.33, $P < 0.001$). The heterogeneity between studies was significant (Q statistic = 313.44, Cochran's Q test, $P < 0.001$; $I^2 = 94.6$), and was not explained by several subgroup analyses. The results of the subgroup analysis based on health status of participants indicated a greater reduction in serum AST levels among patients with liver diseases as compared to patients without liver disease (MD = -17.39, 95% CI: -24.10, -10.76, $P < 0.001$ vs. MD = -4.32, 95% CI: -7.45, -

1.18, $P = 0.007$; **Figure 2B**). Pooled analysis of studies, in which control subjects received placebo or drug, showed a significant reduction, while there was no considerable difference in serum AST levels in studies with non-treated controls. We also observed that L-carnitine supplementation in studies with intervention doses of ≥ 2000 mg/day significantly reduced circulating AST levels compared with control groups (MD = -13.53, 95% CI: -19.43, -7.63, $P < 0.001$; **Figure 3B**), however, there was no significant change when the analysis was done for studies with intervention doses of less than 2000 mg/day. Moreover, the reducing effect of L-carnitine on serum AST levels was seen in both subgroups of duration; however, the effect was lower for studies with a duration of shorter than 12 weeks. **Table 4** details the overall, as well as subgroup analyses, results.

The effect of L-carnitine on other liver enzymes

The pooled estimate of six trials (35-37, 43, 80, 83) evaluating the effect of L-carnitine supplementation on serum GGTP levels showed a significant reduction (MD = -7.88 IU/L, 95% CI: -12.11, -3.64, $P < 0.001$), with no significant between-study heterogeneity (Q statistic = 7.39, Cochran's Q test, $P = 0.193$; $I^2 = 32.4\%$).

Sensitivity analysis and publication bias

The sensitivity analysis revealed that the significant effect of L-carnitine supplementation on circulating ALT and AST levels did not change by removing any of the included trials. The summary effect of the L-carnitine on GGTP levels changed to a non-significant reduction after removing the Lim et al. study (37) (MD = -10.08 IU/L, 95% CI: -20.55, 0.38) and Georgala et al. study (35) (MD = -9.84 IU/L, 95% CI: -21.51, 1.83). We also performed a sensitivity analysis to assess whether results varied by the quality of studies. The results showed that, by excluding the trials that had high risk of bias in overall assessment (37, 38, 41, 78), the

significant reducing effect of L-carnitine supplementation on circulating ALT and AST levels was unaltered.

No evidence of publication bias was found regarding the effect of L-carnitine consumption on ALT (Begg's test, $P = 0.077$; Egger's test, $P = 0.097$) and GGTP (Begg's test, $P = 1.00$; Egger's test, $P = 0.918$) levels. However, visual examination of funnel plot, as well as Begg's and Egger's asymmetry tests (Begg's test, $P = 0.034$; Egger's test, $P = 0.009$), suggested evidence of publication bias for the meta-analysis of serum AST levels. However, using the trim and fill analysis, which conservatively imputes estimates from hypothetical negative unpublished studies, we found the results remained unchanged.

Quality of meta-evidence

The total scores of quality of meta-evidence, which was assessed using the NutriGrade scoring system, were 6.6 for ALT and AST (indicating moderate confidence in the effect estimate, which shows future well designed clinical trials are still needed to confirm our results), and 4.4 for GGTP (indicating low confidence in the effect estimate, which shows further research will provide important evidence on the confidence and likely change the effect estimate).

DISCUSSION

In the present systematic review and meta-analysis, we assessed the efficacy of L-carnitine supplementation in reducing serum liver enzyme levels; by reviewing the available published controlled intervention trials, for the first time. The synthesis of the data confirmed the beneficial effect of L-carnitine intake on decreasing ALT, AST, and GGTP levels. The subgroup analysis showed that L-carnitine might be more effective in reducing liver enzymes when higher doses (≥ 2000 mg/day) are supplemented, when the treatment duration is more than 12 weeks and also when the supplementation is done in patients with liver diseases.

The absorption of oral L-carnitine across the intestinal cells occurs through both active and passive means of transport. These pathways assure the high concentration of L-carnitine in tissues that are dependent on fatty acids oxidation as a fuel source (86, 87). Oral supplementation of L-carnitine (1-6 gr) has been reported to have a biological availability between 5-18% (86), and this limited bioavailability might be associated with metabolization of L-carnitine by gut microbiota prior to absorption (88, 89). This point may be a logical explanation for the efficacy of higher doses of L-carnitine in reducing ALT and AST levels (30). It has been stated that supplementation with L-carnitine is regarded as safe for doses up to 15 g/day in healthy men (42), although doses of 100-400 mg/kg/day is recommended in carnitine deficiency, and, importantly, the L-carnitine dose should be compatible with each patient by measurement of plasma L-carnitine levels. A few side effects, like diarrhea, intestinal problems and the production of trimethylamine, resulting in a fishy odor, have also been observed following high doses of L-carnitine supplementation, which can be effectively treated by reducing the dosage (90).

In the current meta-analysis, we observed a significant reduction in circulating ALT and AST levels following supplementation with L-carnitine in patients with and without liver diseases, however, this reduction was higher in patients with liver diseases compared with other

participants. Indeed, it seems that L-carnitine supplementation elicits a greater beneficial effect for patients who have high levels of liver enzymes at baseline. Since the liver is a major organ responsible for detoxification and metabolization of various compounds that produce reactive oxygen species (ROS), liver diseases might lead to increased ROS production (91, 92). Consequently, oxidative stress induces impairments in mitochondrial β -oxidation (93, 94). The disruption of β -oxidation is a major contributor in the pathogenesis of nonalcoholic fatty liver disease, which causes the accumulation of fatty acids within the hepatocytes and the progression of the disease (95-97). Therefore, the essential role of L-carnitine in the transfer of the long-chain fatty acids inside mitochondria for β -oxidation might be a reason for reducing ALT and AST levels, especially in patients with liver disease (31, 98, 99). Furthermore, it has been proposed that L-carnitine, due to antioxidant and antiradical properties, might be useful in preventing oxidative stress and the activity of enzymes involved in defense reactions against oxidative damage (100). On the other hand, carnitine deficiency is more likely to occur in liver diseases (47, 101), which impairs the mitochondrial β -oxidation of fatty acids, causing acute metabolic decompensation with elevated transaminases, hepatic encephalopathy, hypoketotic hypoglycemia, and cardiomyopathy (102). Accordingly, the correction of carnitine deficiency by oral supplementation of L-carnitine might be beneficial in these patients (101).

There are a number of limitations in the present meta-analysis, although some of them are associated with inherent shortcomings of clinical trials such as heterogeneous methodological approaches regarding the characteristics of participants (for instance, changes in carnitine metabolism in uremia and its depletion is expected in hemodialysis patients), L-carnitine dosage (500-4000 mg/d), and type of control group (placebo, drug or without treatment). Moreover, the duration of L-carnitine supplementation varied from study-to-study, therefore further studies are needed to examine the time-dependent effect of L-carnitine supplementation in reducing ALT and AST levels. Also, a number of the included studies did not explain the

method used to assess the adherence to the treatment, for instance, pill counts or measurement of serum carnitine (37, 39, 42, 43, 77, 78). On the other hand, the bioavailability of L-carnitine was not assessed in the included studies, so the amount of available L-carnitine in the blood after ingestion is not clearly specified. It is also important to note that these findings should not be generalized to patients with primary or secondary carnitine deficiency (caused by genetic alterations in renal handling or muscle transport of L-carnitine and impaired renal tubular resorption from drug toxicity or hemodialysis, respectively). In contrast to these limitations, there are several strengths in our study. We conducted a comprehensive and systematic search to identify all published studies on this topic. We also performed several subgroup analyses to evaluate the potentially different effects of L-carnitine supplementation caused by the intervention dose and duration, the type of control group, and the health status of the participants. Moreover, the findings of the present meta-analysis were not sensitive to the results of any one of the included studies, which highlights the robustness of the findings. The present systematic review and meta-analysis provides evidence for the beneficial effects of L-carnitine supplementation in reducing serum liver enzyme levels. Our findings also showed that L-carnitine can be more effective among patients with liver diseases and with intervention doses of more than 2000 mg/day. However, since the majority of the included studies were judged to have an “unclear” risk of bias, the authors suggest that further high-quality trials, with an adequate duration and sample size, are conducted to reliably confirm the efficacy and safety of L-carnitine supplementation in improving liver enzymes.

Conflict of Interest

There is no conflict of interest to report for this study.

Contributions of authors

The responsibilities of authors were as follows: ASA, MM, and NRJ developed the search strategy; FP, NT and MM conducted the electronic searches and study selection; FP, NRJ, and MM conducted data extraction and tabulated data; ASA, MM, and NRJ conducted the data analysis and interpretation of results; FP, NRJ, and NT wrote the first draft of the manuscript; ASA and CC revised the manuscript and all authors read and approved its final version.

Funding source

The present study was funded by Nutrition and Food Security research center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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Table 1- The Population, Intervention, Comparison, Outcome, Study types (PICOS) criteria

| Criteria | Description |
|--------------|--|
| Population | Adults aged >18 year |
| Intervention | L-carnitine supplement |
| Comparison | Placebo, without treatment or other drugs/supplements |
| Outcome | Enzymes mainly produced by liver [.alanine aminotransferase (ATL), aspartate aminotransferase (AST), and other possible markers like gamma-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP)] |
| Study types | Controlled clinical trials |

Table 2. Characteristics of controlled clinical trials that were included in the systematic review

| Study, Year (ref) | Country | Number, Sex (F/M)* | Age (year) | Study design | Duration (weeks) | Intervention group | Control group | Reported outcomes | Notes about participants |
|------------------------------|---------|--------------------|-----------------------------------|--------------|------------------|---|-------------------|---------------------|---|
| An et al., 2016 (85) | Korea | 53 F/M | 20-80 Int: 49 Con: 50.9 | Parallel | 12 | Three tablet twice per day, 330 mg L-carnitine in each tablet | Placebo | ALT* AST* | Patients with hypothyroidism on levothyroxine treatment |
| Alavinejad et al., 2016 (79) | Iran | 54 16F/38M | Int: 60 Con: 59 | Parallel | 12 | 750 mg L-carnitine three times day | Placebo | ALT AST | Type 2 diabetic patients with NAFLD* |
| Mosah et al., 2015 (42) | Iraq | 60 F | 20-40 Int: 33.11 Con: 32.72 | Parallel | 12 | 1000 mg/d L-carnitine | Without treatment | ALT AST | Obese women with a BMI* ≥ 30 kg/m ² |
| Hassan et al., 2015 (43) | Japan | 50 12F/38M | Int: 24 Con: 26 | Parallel | 12 | 600 mg/day L-carnitine | Without treatment | ALT AST GGTP* | Patients in intermediate stage hepatocellular carcinoma |
| Higuchi et al., 2014 (41) | Tokyo | 131 F/M | 20-85 Int: 67 Con: 68 | Parallel | 48 | 20 mg/kg/day L-carnitine | Without treatment | ALT AST | Hemodialysis patient |
| Somi et al., 2014 (77) | Iran | 80 66F/14M | 25-62 Int: 40.3 Con: 41.1 | Parallel | 24 | 500 mg/d L-carnitine | Without treatment | ALT AST | Patient with NAFLD |
| Fukami et al., 2013 (40) | Japan | 70 26F/44M | Int: 68 Con: 67 | Parallel | 24 | 900 mg/d L-carnitine | Without treatment | ALT AST | Hemodialysis patient |
| Jun et al., 2013 (78) | Korea | 119 42F/77M | Int: 43 Con: 44.9 | Parallel | 48 | 2472 mg/d L-carnitine + 0.5 mg entecavir | 0.5 mg entecavir | ALT | Hepatitis B patients |

| | | | | | | | | | |
|-----------------------------------|---------|----------------|---------------------------------|----------|----|--|--|--------------------|--|
| Odo et al., 2013(83) | Japan | 21 M | 20-60 Int: 44.4 Con: 40.2 | Parallel | 4 | 500 mg/d L-carnitine | Placebo | ALT AST GGTP | Healthy volunteers (overweight) |
| Mohtadinia et al., 2013 (82) | Iran | 14 M | Int: 20.7 Con: 21.2 | Parallel | 3 | 2000 mg/d L-carnitine | Placebo (2000 mg/d maltodextrin) | AST | Healthy male football players |
| Malagurnera et al., 2011 (38) | Italy | 69 27F/42M | Int: 35 Con: 34 | Parallel | 48 | 2000 mg L-carnitine twice a day + 1.5 µg/kg/wk peg interferon-α 2b + ribavirin | 1.5 µg/kg/wk peg interferon-α 2b + ribavirin | ALT AST | Patients with chronic hepatitis C virus |
| Malaguarnera et al., 2010 (36) | Italy | 74 34F/40M | 28-60 Int: 47.9 Con: 47.8 | Parallel | 24 | 2000 mg/d L-carnitine + 1600-calorie diet | 2000 mg/d Placebo + 1600-calorie diet | ALT AST GGTP | Patient with nonalcoholic steatohepatitis |
| Lim et al., 2010 (37) | Korea | 45 F/M | Int: 29 Con: 16 | Parallel | 12 | 600 mg/d L-carnitine | Without treatment | ALT AST GGTP | Patient with non- alcoholic fatty liver disease |
| Delas et al., 2008 (39) | Croatia | 30 18F/12M | 18-32 Int: 23.1 Con: 21.3 | Parallel | 2 | 2000 mg/d L-carnitine | 2000 mg/d Placebo | ALT AST | Healthy volunteers |
| Malaguarnera et al., 2008 (81) | Italy | 115 47F/68M | Int: 48 Con: 45 | Parallel | 12 | 2000 mg Acetyl-L- carnitine twice daily | Placebo | ALT AST | Cirrhotic patients with minimal hepatic encephalopathy |
| Malaguarnera et al., 2002 (47) | Italy | 70 27F/43M | NR Int: 56.8 Con: 57.7 | Parallel | 24 | 2000 mg/d L-carnitine + 3 million IU Interferon-α three times a week | 3 million IU Interferon-α three times a week | ALT AST | Patient with chronic hepatitis C treated with Interferon-α |

| | | | | | | | | | |
|-------------------------------|--------|----------------|------------------------|------------|---|--|--------------------------|--------------------|---|
| Benvenga et al., 2001(80) | Italy | 10 F | Int: 43.4 Con: 43.4 | Cross-over | 8 | 4000 mg/d L-carnitine + l-thyroxine | Placebo + l-thyroxine | ALT AST GGTP | Thyroid patients |
| Georgala et al., 1999 (35) | Athene | 40 F/M | NR | Parallel | 6 | 100 mg/kg/day L- carnitine | 100 mg/kg/day Placebo | ALT AST GGTP | Patient with cystic acne on isotretinoin therapy |
| Singh et al, 1996 (84) | India | 101 10F/91M | Int: 49.2 Con: 50.5 | Parallel | 4 | 2000 mg/d L-carnitine | Placebo | AST | Patients with suspected acute myocardial infarction |

*ALT, alanin aminotransferase; AST, aspartat aminotransferase; GGTP, gamma-glutamyl transpeptidase; Con, control; F, female; Int, intervention M, male; NR, not reported;
NAFLD, non-alcoholic fatty liver disease

Table 3. Risk of bias assessment according to the Cochrane collaboration's risk of bias assessment tool

| Study, Year (ref) | Random sequence generation | Allocation concealment | Blinding of participants and personnel | Blinding of outcome assessment | Incomplete outcome data | Selective reporting | Summary of overall assessment |
|-------------------------------|---|-----------------------------------|---|---|------------------------------------|--------------------------------|--|
| An et al., 2016 (85) | Low | Low | Low | Unclear | Low | Low | Unclear |
| Alavinejad et al., 2016 (79) | Low | Unclear | Low | Unclear | Low | Low | Unclear |
| Mosah et al., 2015 (42) | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Unclear |
| Hassan et al., 2015 (43) | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Unclear |
| Higuchi et al., 2014 (41) | Low | High | High | Unclear | Low | Low | High |
| Somi et al., 2014 (77) | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Unclear |
| Fukami et al., 2013 (40) | Unclear | Unclear | Unclear | Unclear | Low | Low | Unclear |
| Jun et al., 2013 (78) | Low | High | High | Unclear | Low | Low | High |
| Odo et al., 2013 (83) | Unclear | Unclear | Low | Unclear | Low | Low | Unclear |
| Mohtadinia et al., 2013 (82) | Low | Low | Low | Unclear | Low | Low | Unclear |
| Malagurnera et al., 2011 (38) | Low | High | High | Unclear | Low | Low | High |
| Malagurnera et al., 2010 (36) | Low | Unclear | Low | Low | Low | Low | Unclear |
| Lim et al., 2010 (37) | High | High | High | Unclear | Unclear | Low | High |
| Delas et al., 2008 (39) | Unclear | Unclear | Low | Unclear | Low | Low | Unclear |
| Malagurnera et al., 2008 (81) | Unclear | Unclear | Low | Unclear | Low | Low | Unclear |
| Malagurnera et al., 2002 (47) | Low | Unclear | Unclear | Unclear | Low | Low | Unclear |
| Benvenga et al., 2001 (80) | Unclear | Unclear | Low | Unclear | Unclear | Low | Unclear |
| Georgala et al., 1999 (35) | Unclear | Unclear | Unclear | Unclear | Unclear | Low | Unclear |
| Singh et al., 1996 (84) | Unclear | Unclear | Low | Unclear | Low | Low | Unclear |

Table 4- The effect of L-carnitine supplementation on serum ALT and AST levels based on several subgroups as well as all studies, using a random-effects model.

| | | | Meta-analysis | | Heterogeneity | | | |
|-----------------------|--------------|----------------|--------------------------------------|----------|----------------|-------------------|--------------------|--------------------|
| | Trials, n | Subjects, n | Weighted mean difference (95% CI) | P effect | Q statistic | P within group | I ² (%) | P between group |
| ALT (IU/L) | | | | | | | | |
| Health status | | | | | | | | |
| Liver disease | 9 | 676 | -20.25 (-31.22, -9.28) | <0.001 | 143.59 | <0.001 | 94.4 | <0.001 |
| Without liver disease | 8 | 415 | -2.84 (-5.33, -0.34) | 0.026 | 50.71 | <0.001 | 86.2 | |
| Control type | | | | | | | | |
| Without treatment | 6 | 436 | -8.85 (-17.74, 0.04) | 0.051 | 260.51 | <0.001 | 98.1 | <0.001 |
| Placebo | 8 | 397 | -10.19 (-18.97, -1.42) | 0.023 | 296.38 | <0.001 | 97.6 | |
| Drug | 3 | 258 | -23.51 (-37.35, -9.68) | 0.001 | 2.35 | 0.309 | 14.9 | |
| Dosage of L-carnitine | | | | | | | | |
| < 2000 mg/d | 7 | 457 | -7.52 (-15.54, 0.49) | 0.066 | 261.58 | <0.001 | 97.7 | <0.001 |
| ≥ 2000 mg/d | 10 | 634 | -14.08 (-22.72, -5.44) | 0.001 | 297.39 | <0.001 | 97 | |
| Duration | | | | | | | | |
| ≤ 12 weeks | 10 | 478 | -9.59 (-17.54, -1.64) | 0.018 | 523.25 | <0.001 | 98.3 | 0.099 |
| > 12 weeks | 7 | 613 | -12.77 (-20.63, -4.90) | 0.001 | 80.37 | <0.001 | 92.5 | |
| Overall | 17 | 1091 | -10.97 (-16.64, -5.48) | <0.001 | 606.33 | <0.001 | 97.4 | - |
| AST (IU/L) | | | | | | | | |
| Health status | | | | | | | | |
| Liver disease | 8 | 557 | -17.39 (-24.10, -10.67) | <0.001 | 36.57 | <0.001 | 80.9 | <0.001 |
| Without liver disease | 10 | 530 | -4.32 (-7.45, -1.18) | 0.007 | 119.94 | <0.001 | 92.5 | |
| Control type | | | | | | | | |
| Without treatment | 6 | 436 | -3.91 (-9.19, 1.36) | 0.146 | 79.39 | <0.001 | 93.7 | <0.001 |
| Placebo | 10 | 512 | -10.77 (-16.37, -5.18) | <0.001 | 204.09 | <0.001 | 95.6 | |
| Drug | 2 | 139 | -29.10 (-43.66, -14.53) | <0.001 | 0.49 | 0.482 | 0 | |
| Dosage of L-carnitine | | | | | | | | |
| < 2000 mg/d | 7 | 457 | -3.14 (-7.72, 1.43) | 0.179 | 81.27 | <0.001 | 92.6 | <0.001 |
| ≥ 2000 mg/d | 11 | 630 | -13.53 (-19.43, -7.63) | <0.001 | 204.42 | <0.001 | 95.1 | |
| Duration | | | | | | | | |
| ≤ 12 weeks | 12 | 593 | -8.62 (-13.06, -4.18) | <0.001 | 253.37 | <0.001 | 95.7 | 0.604 |
| >12 weeks | 6 | 494 | -10.82 (-19.16, -2.47) | 0.011 | 59.81 | <0.001 | 91.6 | |
| Overall | 18 | 1087 | -9.03 (-12.73, -5.33) | <0.001 | 313.44 | <0.001 | 94.6 | - |
| GGTP (IU/L) | | | | | | | | |
| Overall | 6 | 240 | -7.88 (-12.11, -3.64) | <0.001 | 7.39 | 0.193 | 32.4 | - |

Figure Legends

Figure 1- The detailed steps of the study selection process.

Figure 2- Forest plots of controlled trials examining the pooled effects of L-carnitine, based on the health status of participants (liver disease/without liver disease) on serum levels of alanine aminotransferase (ALT) (A), and aspartate aminotransferase (AST) (B).

Figure 3- Forest plots of controlled trials examining the pooled effects of L-carnitine, as well as based on the intervention dose of supplementation (< 2000 mg/d/ ≥ 2000 mg/d) on serum levels of on serum levels of alanine aminotransferase (ALT) (A), and aspartate aminotransferase (AST) (B).