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The Effect of L-carnitine Supplementation on Liver Function, Folate and Vitamin B_{12} Levels in Patients with Type 2 Diabetes Mellitus: A Randomized, Double-Blind, Clinical Trial

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ABSTRACT

Background: Carnitine is necessary for allowing the long-chain fatty acids to pass the inner mitochondrial membrane to induce β -oxidation. Lack of carnitine and abnormalities of mitochondria play an important role in forming fatty deposition in the liver, and hence, developing steatohepatitis. Carnitine and acylcarnitine identified in human erythrocytes and intra-erythrocyte acetylcarnitine have a significant relationship with the plasma levels. **Methods**: The present study was conducted to investigate the possible effects of L-carnitine on liver function, folate and vitamin B12 levels in patients with type 2 diabetes mellitus (T2DM). In this study, 70 patients with T2DM were randomly assigned to either a L-carnitine (CG) and a placebo group (PG). For 12 weeks, the first group received 1000 mg/d oral L-carnitine, whereas the second group received 1000 mg/day wheat starch as placebo. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), folate, Vitamin B12, complete blood count (CBC) including blood cells and indicators related to anemia were assessed at baseline. **Results**: 64 patients managed to complete the study (32 in each group). The results indicated that consumption of L-carnitine compared with placebo had no significant effect on liver enzymes, folate, vitamin B12, and CBC with differential. **Conclusions**: Daily intake of 1000 mg L-carnitine for 12 weeks had no effect on liver function and anemia indicators including CBC, folate, and vitamins B12.

Keywords: *Carnitine; Diabetes mellitus; Liver; Vitamins; Clinical trial*

Introduction

Diabetes mellitus (DM) is a common chronic metabolic disorder with an increasing prevalence. It has become a major public health concern, leading to serious disorders in the body (Aekplakorn *et al.*, 2011, Meo, 2009). In 2019, the International Diabetes Federation (IDF) estimated

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that approximately 463 million people aged 20-79 worldwide suffer from DM. This will increase to 578 million in 2030 and 700 in 2045 with type 2 diabetes (T2DM) accounting for about 90% of all cases. In 2019, the number of people with diabetes in Iran at the age range of 20-79 was reported to be 5.4 million with a prevalence of 9.4%(International Diabetes Federation (IDF), 2019).

T2DM is associated with other chronic diseases and is influenced by genetic factors, obesity, poor diet and physical inactivity. This ultimately leads to anatomical, structural and functional changes in several important organs of the body (Galicia-Garcia *et al.*, 2020, Sami *et al.*, 2017). Further progression of the disease is associated with cardiovascular complications (Kayama *et al.*, 2015), nephropathy, retinopathy and neuropathy (Vinik *et al.*, 2013). The prevalence of non-alcoholic fatty liver disease (NAFLD) in patients with T2DM was found to be high (68.1% of general population), and diabetes, obesity, and hyperlipidemia are considered important risk factors for NAFLD (Mohamed *et al.*, 2022). There is also evidence that liver enzymes and NAFLD act as useful biomarkers for diabetes (Atiba *et al.*, 2013). Moreover, alanine aminotransferase/ aspartate aminotransferase (ALT/AST) ratio may be one of the best markers for insulin resistance (Zhao *et al.*, 2017). Higher levels of AST, gamma-glutamyl transferase (GGT), and ALT have also been shown to be associated with increased HOMA-IR index and the risk of T2DM (Sheng *et al.*, 2018, Shibabaw *et al.*, 2019). Oxidative stress and chronic inflammation are among the disorders that can lead to progression of the disease, and higher level of serum GGT was independently associated with increased load of subclinical inflammation across metabolic states (Ali *et al.*, 2016, Turgut and Tandogan, 2011).

Plasma levels of vitamin B12 and folate have been less reported in patients with T2DM compared to non-diabetic patients (Ebesunun and Obajobi, 2012). Vitamin B12 deficiency contributes to accumulation of methyl-malonic acid. This causes oxidative stress, impairs mitochondrial function, disrupts cellular energy metabolism, triggers cell death, and ultimately produces more inflammatory cytokines such as alpha tumor necrotizing factor (TNF-α) (Chen *et al.*, 2008, Liu *et al.*, 2022). In addition, these deficiencies trigger disruption of methionine synthesis and homocysteine (Hcy) production as a potential risk factor for cardiovascular disease associated with oxidative stress in diabetics (Al-Maskari *et al.*, 2012, Kolling *et al.*, 2011). Vitamin B12 and folate deficiency cause anemia. Consequently, the serum level of these vitamins has been properly determined and defined to prevent anemia (Fenech, 2012). Evidence also suggests that deficiency of vitamin B12 is associated with acyl carnitine accumulation, indicating high intracellular levels of acyl-CoA. Moreover the deficiency leads to accumulation of propionyl carnitine (Brass and Stabler, 1988, Sarafoglou *et al.*, 2011). There is a hypothesis that propionyl carnitine is a major product of propionate metabolism regarding vitamin B12 deficiency and the optimal rate of propionyl carnitine formation depends on availability of external carnitine. This has been investigated and proven in studies by Brass (Brass and Ruff, 1989, Brass and Stabler, 1988).

Inadequate exogenous intake, malabsorption and lack of endogenous hepatic synthesis of L-carnitine can lead to its deficiency in the body, which is accompanied by liver cirrhosis (Cave *et al.*, 2008). L-Carnitine affects intracellular reactions by limiting the oxidative stress process, which ultimately reduces response of inflammatory mediators and improves the outcomes of nonalcoholic steatohepatitis (NASH) (Romano *et al.*, 2008). L-carnitine supplementation improves body composition and levels of liver enzymes, having a direct effect on liver function (Pirmadah *et al.*, 2019, Talenezhad *et al.*, 2020b). In addition, it plays a key role in elimination of short and medium chain fatty acids, regulation of CoA/CoA acyl ratio within mitochondria, transmission and oxidation of long chain fatty acids, glucose metabolism, detoxification of toxic metabolites and cell wall

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stabilization (di San Filippo *et al.*, 2008, Malaguarnera *et al.*, 2010). The supplementary effect of L-carnitine on blood cells has been investigated in many studies (Strasser *et al.*, 2007, Sweeney and Arduini, 2004). The role of L-carnitine in the management of anemia may improve and maintain red blood cell survival by augmenting the stability of erythrocyte membranes (Arduini *et al.*, 1993). A study conducted by Bonomini et al. revealed that L-carnitine-enriched peritoneal dialysis solutions have demonstrated nti-anemia action due to its effects on erythropoiesis, and positive effects on the longevity and deformability of erythrocytes (Bonomini *et al.*, 2019). Moreover, in a clinical trial study, it was identified that treatment with L-carnitine during a period of 24 months may be effective for reducing muscle cramping and improving hemoglobin levels in dialysis patients (Kuwasawa-Iwasaki *et al.*, 2020). In some studies, the beneficial effects of L-carnitine on upgrading the survival time of red blood cells in treated patients could not be observed (Kletzmayr *et al.*, 1999). L-Carnitine stabilizes erythrocyte membranes by increasing Hct and improving cellular sodium, potassium and adenosine triphosphate through reducing their plasma inhibition (Donatelli *et al.*, 1987, Labonia *et al.*, 1987). It also protects red blood cells against toxicity above the level of intracellular calcium (Agroyannis *et al.*, 2002).

To evaluate the clinical effect of L-carnitine in the treatment of complications of T2DM, especially elevated blood Hcy levels, the researchers conducted a large randomized, double-blind, and placebo-controlled study (Talenezhad *et al.*, 2020a, Talenezhad *et al.*, 2020c). Considering the role of folate and vitamin B12 in Hcy metabolism, their deficiency in diabetic patients can be associated with increased Hcy levels (Fotiou *et al.*, 2014), as well as the prevalence NAFLD (Raza *et al.*, 2021). As part of a larger study, it was decided to investigate the effect of L-carnitine supplementation on patients' liver function and anemia indices including vitamins B12 and folate.

Materials and Methods

Study design and participants: This was a randomized double-blind, clinical trial. Seventy adults with T2DM in the age range of 41–75 years were recruited from Yazd Diabetic Research Center. Patients met the following inclusion criteria: aging 30 and above, having a body mass index (BMI) of greater than 25 kg/m^2, and HbA1c of lower than 10%, having no clinical diagnosis of chronic gastrointestinal, liver or kidney disease, untreated hypothyroidism, hypertension, systemic infection, gangrene, having a history of myocardial infarction, unstable angina, severe arrhythmia, and cardiac surgery in the last 3 or 6 months, no current use of antibiotics or other prescribed medicine, no use of L-carnitine supplements and group B vitamins over the last 3 months and antioxidant intake (vitamins A, D, C and E) within 1 month of the initiation of the experimental periods, no specific dietary practice for weight loss in the past year. Patients were excluded if they could not comply with or follow the study protocol. Those who showed no desire to continue with the trial for any reason were not included.

The patients were randomly allocated to two groups of thirty-five; the first group received L-carnitine 1000 mg/d (LG) divided into 2 equal doses of one 500 mg tablet after breakfast and during dinner for 12 weeks (L-carnitine, Karen Pharmaceutical and Nutrilife Co., Yazd, Iran). The second group received placebo (PG) with the same dose, shape, color and duration (placebo, Karen Pharmaceutical and Nutrilife Co. Yazd, Iran).

Allocation concealment and compliance with the intervention: With an allocation ratio of 1:1, patients were randomly assigned into two groups through a computer-generated randomization sequence. Researchers and participants involved in data collection and analysis were blinded to the randomization and treatment allocation until the final analyses were completed. The matching placebo capsule was identical to L-carnitine capsule in terms of appearance, taste, and smell. L-carnitine and placebo compliance was assessed by counting the number of remaining capsules at their

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subsequent visit.

Measurements: Once the patients had fasted for 10-12 hours, 7 to 10 ml of venous blood samples were obtained, and the serum was separated from clotted blood by centrifugation and transferred into three serum separator tubes. Serum samples were stored at −70 °C until the end of the study period. Then, ALT, AST, ALP, GGT were measured in fasting serum using biochemical kits from Pars Azmon (Tehran, Iran). In addition, folate (ng/ml) and vitamin B12 (pg/l) were determined using the ELISA kits (Monobind, USA). Erythrocyte count (RBC, 10^6/µl), leukocyte count (WBC, 10^3/µl), hemoglobin content (Hb, g/dl), Hct (%), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dl) and platelet count (10^3/µl) were assessed with the routine clinical chemistry procedures using commercial kit. Patients were asked to maintain their usual diet and level of physical activity during the study period. All the subjects completed the 3-day food records and physical activity records (in a week's time) in weeks 0 and 12 of the intervention. Daily macro- and micro-nutrient dietary intakes were also analyzed by nutritionist IV software. The international physical activity questionnaire (IPAQ) was used to estimate physical activity based on the metabolic equivalent of task (MET) score.

Ethical considerations: The ethics committee affiliated with Yazd Shahid Sadoughi University of Medical Sciences (Ethics code: IR.SSU.SPH.REC.1397.067) approved the study protocol. Moreover, this trial was registered in the Iranian Clinical Trial Registration Center (www.irct.ir) with the code IRCT2017100936681N. Informed consent was obtained from the participants before initiation of the study.

Data analysis: Data analysis was carried out using SPSS-24 (version 24; SPSS Inc., Chicago Illinois, USA). The results indicated in the manuscript and tables are reported as mean ± standard error (SE) or 95% confidence intervals (CIs). A P-value of < 0.05 was also considered statistically significant. Kolmogorov-Smirnov and Shapiro-Wilk tests followed by test graphs and numbers related to Skewness and Kurtosis were used to examine the normal distribution of the quantitative data. The paired t-test was also employed to evaluate within-group differences (before and after intervention) regarding normal data. To identify any differences in the baseline variables and at the end of the study and to compare the mean changes in the intervention group's independent samples, student t-test was deployed. Moreover, the ANCOVA test was carried out to detect any differences between the intervention groups at the end of the experiment by adjusting baseline values.

Results

Baseline characteristics of participants: 70 participants were randomly assigned to the intervention group, of whom 64 completed the trial. Six participants withdrew during the study. In the LG, two participants dropped out because of their lack of interest to continue and use of antioxidant supplementation (n=2). Moreover, one subject dropped out because of concern for gastrointestinal side effects following supplementation with L-carnitine (n=1). In the PG, one patient reported renal complications following placebo (n=1) and two patients were excluded for lumbar disc surgery and loss of interest (**Figure 1**). A summary of the baseline characteristics of all the participants is provided in **Table 1**. The mean age of the total sample was 55.67 ± 0.99 ranging from 41 to 75 in each treatment group. 61% were women, 73% of whom were housewife. There were no significant differences between groups in terms of all baseline characteristics ($P > 0.05$). The patients in LG and PG were compared for other diseases, for which no significant difference were identified between the groups ($P = 0.606$).

Physical activity, energy and nutrient intake: Energy intake, macronutrients and some micronutrients (based on a 3-day diet) as well as the level of physical activity (in a period of one week) are presented in **Table 2**. After adjusting for baseline values, the comparative analysis

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demonstrated no statistically significant difference regarding some of the micronutrient and macronutrient intake, energy and level of physical activity between LG and PG ($P > 0.05$).

Effect of L-carnitine on blood and serum parameters: After 12 weeks of treatment with L-carnitine, independent samples t-test failed to detect any statistically significant difference in terms of changes in serum levels of vitamin B_{12}, folate and liver biomarkers compared to the PG. The changes also did not appear to be considerable for CBC, which included blood cells and indicators related to anemia. In the final analysis, to achieve a more accurate and valuable P-value and identify the differences between the treatment groups, results of ANCOVA test were reported with adjustment for baseline values of the target variables. The results of ANCOVA test did not show any statistical significance for serum levels of ALT (P=0.302), AST (P=0.061), ALP (P=0.546), GGT (P=0.312), vitamin B_9 (P=0.343), vitamin B_{12} (P=0.606) as well as RBC (P=0.050), WBC (P=0.666), Hb (P=0.355), Hct (P=0.140), MCV (P=0.523), MCH (P=0.928), MCHC (P=0.481) and platelet count (P=0.832).

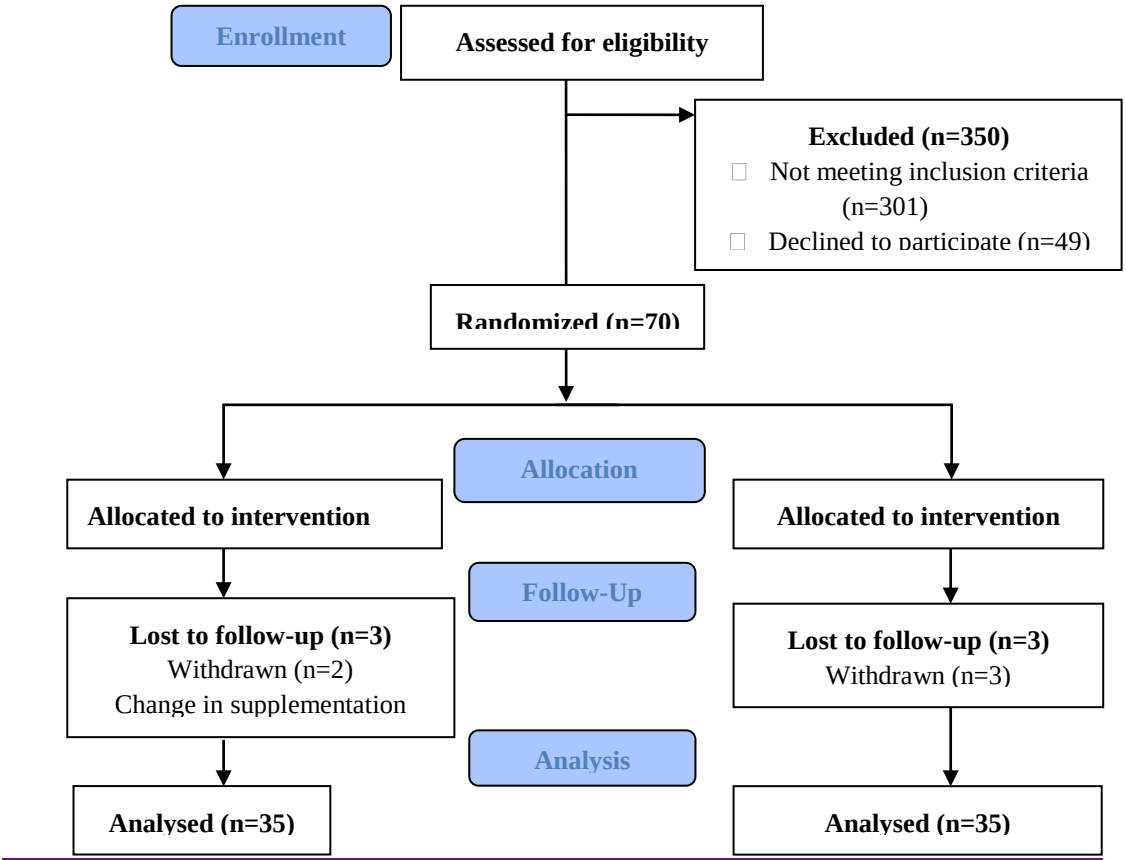

Figure1. Consort flow chart diagram of sampling and intervention at the present study.

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Table 1. baseline characteristics of study participants.

Variables/ Groups	L-carnitine group (n=35)	Placebo group (n=35)	P-value[b]
Age (year)	56.40±1.49	54.90±1.32	0.469
Height (cm)	162.89±1.95	160.89±1.47	0.418
Weight (kg)	76.03±2.03	77.22±1.99	0.678
Body mass index (kg/m^2)	28.63±0.56	29.54±0.74	0.333
Hemoglobin A1c (%)	6.90±0.14	7.00±0.16	0.642
Duration of diabetes (year)	4.91±0.64	4.48±0.80	0.678
Physical activity (Met/min/day)	1863.69±49.32	1811.66±44.14	0.435
Sex			
Male	16(45.7)	11(31.4)	0.220
Female	19(54.3)	24(68.6)	
Status of vitamin B_{12}			
Normal	5(14.3)	2(5.7)	0.232
Deficiency	30(85.7)	33(94.3)	
Status of vitamin B_9			
Normal	2(5.7)	1(2.9)	0.550
Deficiency	33(94.3)	34(97.1)	
Job			
Housewife	17(48.6)	20(57.1)	0.350
Retired	6(17.10)	9(25.7)	
Free job	8(22.9)	3(8.6)	
Employee	4(11.4)	3(8.6)	
Education			
Illiterate	2(5.7)	1(2.9)	0.661
Elementary	11(31.4)	14(40)	
Diploma	16(46.6)	13(37.1)	
Bachelor	3(8.6)	6(17.1)	
Above bachelor	3(8.6)	1(2.9)	
Drug used			
Metformin	24(72.7)	21(67.7)	0.663
Acarbose	11(33.3)	12(40.0)	0.583
Diabetes	9(27.3)	13(41.9)	0.217
Ziptin	7(21.2)	8(26.7)	0.612
Zipmet	5(16.1)	10(32.3)	0.138
Diamicron	9(27.30)	4(12.9)	0.153
Aspirin	8(25.0)	9(30.0)	0.659
Anti- hypertensive	15(45.5)	14(45.2)	0.981
Statins	18(55.5)	22(71.0)	0.175
Other disease			
Hypothyroidism	4(11.4)	2(5.7)	0.606
Anemia	0	2(5.7)	
Waist and Neck Disc	1(2.9)	1(2.9)	
Slight fatty Liver	1(2.9)	1(2.9)	
Rheumatism	0	2(5.7)	
Pemphigus	1(2.9)	0	

[a]:Mean ± SE; [b]: P-value resulted from independent t-test for quantitative and Chi-square for qualitative variables between the two groups.

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Table 2. Daily nutrient intake based on a 3-day diet in patients with T2DM.

Variables	L-carnitine group (n=35)	Placebo group (n=35)	P-value[a]
Energy (kcal/day)			
Before	1978.65±53.93[b]	1957.60±46.85	0.144
After	1963.60±52.14	1902.45±47.67	0.122
Change	-15.05±22.93	-55.15±30.50	0.248
P-value[c]	0.516	0.080	
Carbohyderate (g/day)			
Before	270.20±7.73	258.83±9.81	0.075
After	269.28±8.49	259.92±9.10	0.165
Change	-0.92±5.57	1.08±6.39	0.926
P-value	0.874	0.866	
Protein (g/day)			
Before	79.11±2.93	76.82±2.44	0.205
After	79.36±2.57	74.47±2.47	0.190
Change	0.25±2.45	-2.35±2.43	0.220
P-value	0.919	0.340	
Total Fat (g/day)			
Before	67.92±2.88	70.76±2.48	0.499
After	67.38±3.22	65.21±2.63	0.804
Change	-0.53±2.15	-5.55±2.71	0.218
P-value	0.804	0.049	
Cholestrol (mg/day)			
Before	262.04±22.07	312.15±24.49	0.147
After	263.29±24.52	249.48±21.87	0.680
Change	1.24±26.67	-62.67±29.13	0.395
P-value	0.963	0.039	
Total dietary fiber (g/day)			
Before	16.62±0.98	16.81±0.88	0.503
After	15.98±0.88	17.29±0.86	0.290
Change	-0.63±1.09	0.48±0.81	0.276
P-value	0.564	0.559	
Selenium (mg/day)			
Before	0.06±0.004	0.06±0.004	0.720
After	0.06±0.004	0.06±0.003	0.384
Change	0.0017±0.004	0.0016±0.003	0.320
P-value	0.700	0.691	
Zinc (mg/day)			
Before	8.15±0.35	8.29±0.34	0.905
After	7.9±0.32	7.88±0.40	0.613
Change	-0.24±0.32	-0.40±0.39	0.836
P-value	0.505	0.309	
Vitamin E (mg/day)			
Before	10.70±1.12	10.33±1.25	0.893
After	9.77±1.47	9.56±1.11	0.718
Change	-0.93±1.48	-0.76±1.00	0.995
P-value	0.535	0.451	
Vitamin A (RE)			
Before	4.07±0.34	4.37±0.32	0.315
After	4.32±0.32	3.87±0.22	0.220
Change	0.25±0.40	-0.49±0.34	0.185
P-value	0.537	0.157	
Vitamin C (mg/day)			
Before	63.10±6.97	77.63±8.11	0.282

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Table 2. Daily nutrient intake based on a 3-day diet in patients with T2DM.

Variables	L-carnitine group (n=35)	Placebo group (n=35)	P-value[a]
After	62.90±6.19	67.47±7.61	0.790
Change	-0.19±8.61	-10.15±6.11	0.825
P-value	0.982	0.106	
Physical activity (Met/min/day)			
Before	1863.69±49.32	1811.66±44.14	0.511
After	1858.77±56.11	1825.43±54.36	0.664
Change	-4.91±29.55	13.77±22.20	0.583
P-value	0.869	0.539	

[a]: Obtained from **ANCOVA**, adjusted for baseline values; [b]: Mean ± SE; [c]: Paired t-test.

Table 3. Within and between comparison mean of hematologic parameters in two groups.[1]

Variables	L-carnitine group (n=35)	Placebo group (n=35)	P-value[a]
Alanine transaminase (u/l)			
Before	23.34±1.51[b]	21.36±1.25	0.322
After	24.44±1.62	21.40±1.33	0.154
Change	1.09±1.45	0.03±1.14	0.302
P-value[c]	0.456	0.976	
Aspartate transaminase (u/l)			
Before	19.01±0.87	18.76±0.98	0.849
After	20.15±1.13	17.87±0.69	0.100
Change	1.13±0.92	-0.88±0.77	0.061
P-value	0.229	0.262	
Alkaline phosphatase (u/l)			
Before	205.86±8.05	202.54±9.91	0.796
After	199.86±7.04	201.29±9.25	0.903
Change	-6±4.82	-1.25±5.26	0.546
P-value	0.223	0.813	
Gamma-glutamyltransferase (u/l)			
Before	19.07±1.38	20.93±1.66	0.395
After	18.69±1.21	21.43±1.77	0.209
Change	-0.38±0.81	0.50±83	0.312
P-value	0.645	0.546	
Vitamin B12 (pg/l)			
Before	340.90±27.66	321.33±18.52	0.561
After	328.89±2419	306.84±22.60	0.508
Change	-12.01±28.25	-14.49±22.96	0.643
P-value	0.673	0.532	
Vitamin B9 (ng/ml)			
Before	12.48±1.03	13.30±1.07	0.586
After	13.86±0.84	13.08±0.96	0.544
Change	1.38±1.26	-0.22±0.72	0.343
P-value	0.283	0.764	
Homocysteine (µmol/l)			
Before	14.43±0.79	14.81±0.96	0.764
After	15.30±0.79	14.23±0.86	0.336
Change	0.87±0.75	-0.57±0.75	0.170
P-value	0.252	0.452	

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Table 3. Within and between comparison mean of hematologic parameters in two groups.[1]

Variables	L-carnitine group (n=35)	Placebo group (n=35)	P-value[a]
White blood cells (10^3/µl)			
Before	7.01±0.27	6.95±0.27	0.865
After	6.93±0.20	6.79±0.24	0.680
Change	-0.08±0.18	0.15±0.17	0.666
P-value	0.648	0.384	
Red blood cells (10^6/µl)			
Before	4.82±0.09	4.80±0.08	0.853
After	4.82±0.10	4.67±0.07	0.220
Change	0.0±0.04	-0.13±0.05	0.048
P-value	0.982	0.023	
Hemoglobin(g/dl)			
Before	14.14±0.22	13.53±0.28	0.102
After	14.34±0.23	13.63±0.28	0.058
Change	0.20±0.13	0.10±0.15	0.355
P-value	0.136	0.511	
Hematocrit (%)			
Before	40.77±0.64	39.90±0.75	0.387
After	39.51±0.63	38.01±0.67	0.113
Change	-1.25±0.36	-1.88±0.48	0.140
P-value	0.002	<0.001	
Mean corpuscular volume (fl)			
Before	85.40±0.81	84.67±0.94	0.564
After	83.99±0.81	82.97±0.88	0.408
Change	-1.41±0.53	-1.69±0.41	0.523
P-value	0.012	<0.001	
Mean corpuscular hemoglobin (pg)			
Before	29.80±0.39	29.24±0.35	0.293
After	30.56±.35	30.08±0.40	0.380
Change	0.75±0.29	0.84±0.20	0.928
P-value	0.017	<0.001	
Mean corpuscular hemoglobin concentration (g/dl)			
Before	34.70±0.35	34.12±0.28	0.190
After	36.25±0.20	35.83±0.27	0.215
Change	1.54±0.32	1.71±0.31	0.481
P-value	<0.001	<0.001	
Platelet (10^3/µl)			
Before	225.56±8.54	252.64±9.46	0.038
After	219.34±7.74	236.38±8.18	0.172
Change	-6.21±6.78	-16.26±4.64	0.832
P-value	0.366	0.001	

[a]: Obtained from **ANCOVA**, adjusted for baseline values; [b]: Mean ± SE; [c]: Paired t-test.

Discussion

L-carnitine administration for 12 weeks failed to significantly affect liver enzymes (ALT, AST, ALP, and GGT), folate and vitamin B12 in patients with T2DM. In addition, the results were not significant for CBC.

To date, no clinical trial study has investigated the effect of L-carnitine on folate and vitamin B12 levels in various diseases. Moreover, few studies have considered the effect of this supplement on liver function in patients with T2DM. There is still insufficient evidence to investigate the effect of L-carnitine on anemia-related factors in animal and human models. In the current trial, the researchers

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did not observe any significant reduction in circulating liver enzymes' levels following supplementation with l-carnitine in patients with T2DM.

This is consistent with several clinical trials which indicated L-carnitine had no significant effect on the level of serum liver enzyme (An *et al.*, 2016, Hassan *et al.*, 2015, Higuchi *et al.*, 2014). Hassan *et al.* found no significant changes in the level of liver enzyme among intermediate-stage hepatocellular carcinoma (HCC) patients after supplementation with 600 mg of L-carnitine over 12 weeks (Hassan *et al.*, 2015). Another clinical trial study conducted in 2016 identified that oral treatment of L-carnitine in patients with hypothyroidism on levothyroxine treatment failed to significantly impact the serum concentration of liver enzyme (An *et al.*, 2016). However, a number of trials provided evidence that supplementation with L-carnitine might be effective in reducing these enzymes (Alavinejad *et al.*, 2016, Malaguarnera *et al.*, 2011, Somi *et al.*, 2014). Some of these studies evaluated the effect of L-carnitine on liver function and treatment of NAFLD (Alavinejad *et al.*, 2016, Somi *et al.*, 2014) and other liver diseases such as NASH (Malaguarnera *et al.*, 2010) and chronic hepatitis C (Malaguarnera *et al.*, 2011). Some desired results have been obtained from the impact of L-carnitine on elevating liver enzymes. Therefore, evidence confirms that L-carnitine can improve abnormally elevated levels of liver enzymes in these patients. However, evidence which runs counter to the study results is based on the fact that diseases that impair liver function can trigger accumulation of toxins in the body and increase production of reactive oxygen species (ROS) (Muriel and Gordillo, 2016). Then, oxidative stress produced by oxidants contributes to disorders of mitochondrial β oxidation (Santos *et al.*, 2013). Deactivation of beta oxidation pathway or carnitine deficiency induce accumulation of fatty acids within the hepatocytes and the progression of the NAFLD (Rolo *et al.*, 2012, Rudman *et al.*, 1977). Due to the fact that L-carnitine is a key component in beta oxidation of long chain fatty acids in mitochondria and also bears antioxidant and anti-radical properties, it is expected to reduce liver enzymes, especially in liver diseases (Indiveri *et al.*, 2011). This study, however, examined liver function in patients with diabetes, not liver disease. A meta-analysis focused on serum levels of enzymes mainly produced by liver, indicated that L-carnitine can have a significant effect on these enzymes, and finally, positively affect liver function especially in patients with liver diseases. Another finding of this study revealed that L-carnitine can be more effective in healthy people or patients who receive intervention doses of higher than 2000 mg/day (Pirmadah *et al.*, 2019). Therefore, one of the reasons for not achieving a significant result in the present study could be related to the low dose of L-carnitine intervention (1000 mg/day).

The intake of 1000 mg/d of L-carnitine does not affect folate serum concentrations, vitamin B12, and CBC including blood cells and indicators related to anemia. It should be noted that the aim of investigating the effect of L-carnitine supplementation on these vitamins was to determine and control their relationship with anemia indicators in the present study and Hcy research (Talenezhad et al., 2020c). There has been no clinical trial on the effect of L-carnitine supplementation regarding these vitamins. Monitoring of vitamins was of great importance in this study. This is because the plasma levels of vitamin B12 and folate was low in patients with T2DM, which was attributed to either the disease itself or anti-diabetic drugs such as metformin (Kim et al., 2019). In addition, these vitamins played an important role regarding anemia. Several studies, however, examined the effect of L-carnitine supplementation on other blood markers associated with anemia. Kudoh et al. reported that long-term consumption of LC did not have beneficial effect on patients with renal anemia (Kudoh et al., 2014). In another RCT study by Maruyama (Maruyama et al., 2017), no significant difference was indicated between the 2 groups during the study period. Results suggested that levocarnitine

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consumption can decrease the dose of erythropoiesis-stimulating agent (ESA) required in patients with renal anemia regarding hemodialysis, and amend response to ESA therapy (Maruyama et al., 2017). Emami Naini reported that oral L-carnitine supplementation may insignificantly increase hemoglobin, and subsequently, reduce needed erythropoietin dose. This reveals the positive effect of oral L-carnitine on anemia. This study which is in line with ours could not detect a significant increase in hemoglobin levels; yet, it improved anemia (Emami Naini et al., 2012). Orasan et al. indicated that supplementation with 500mg/day L-carnitine during 3 months had no effect on anemia in hemodialysis (HD) patients (Orasan et al., 2011). Contrary to the results of this study, a clinical trial conducted in 2005 demonstrated that a 4-month period of L-carnitine supplementation may improve anemia with a significant increase in the Hct and Hb levels, and reduce the weekly required dose of the rHuEPO (Kadiroglu et al., 2005). Additionally, in 2002, a trial examined the effect of 2000 mg oral L-carnitine supplementation on Hct and Hb levels in patients suffering from end stage renal disease (ESRD) regarding continuous ambulatory peritoneal dialysis (CAPD). It revealed that consumption of L-carnitine can increase Hct and Hb levels and reduce the patient's erythropoietin (rHuEpo) dose per week (Sotirakopoulos et al., 2002). The dose of L-carnitine used was twice, compared with this study. Furthermore, in another study, Trovato detected a rise in hematocrit in HD patients treated with oral L-carnitine for 12 months. Intervention in Trovato's study took much longer than this study (Trovato et al., 1982). Mechanisms which can explain the significant effects of L-carnitine on indicators associated with anemia include augmenting the survival rate of red blood cells. This was done by increasing Na–K pump activity of the erythrocyte membrane, increasing their membrane stability, effecting some enzymes and metabolic functions in the erythrocytes (Arduini et al., 1993, Labonia et al., 1987), raising red blood cell osmotic resistance (Evangeliou and Vlassopoulos, 2003), and protecting them from high toxicity of intracellular calcium (Agroyannis et al., 2002).

This study had several limitations. First, it was performed on diabetic patients without NAFLD or anemia. Moreover, the associated blood parameters were normal, and there was no deficiency. In other words, the studied indicators were not deficient or abnormal, which affected the results. Second, it related to the lack of measurement of carnitine, other parameters of NAFLD, and anemia at the baseline and at the end of the trial. And third, there was a low dose of intervention. If a higher dose was used, more favorable results might have been obtained.

Conclusion

Carnitine consumption fails to reduce the level of liver enzyme, folate, vitamin B_{12}, and also CBC including blood cells and indicators related to anemia in T2DM. This is done without additional interventions like physical activity or low-caloric diets. Further high-quality and large RCT are needed to clarify the actual effect of L-carnitine intake on parameters of liver and anemia in the patients with T2DM.

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Conflicts of interest

The authors declared no conflict of interest.

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Authors' contributions

Mozaffari-Khosravi H involve in designing, data analyzing, supervising and writing the manuscript. Talenezhad N involved in designing, data collecting, data analyzing and writings the manuscript. Akhlaghi M, Raeisi-Dehkordi H and Zarei S participated to laboratory measurements and analyzing the data. All authors read the

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