Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

L-Carnitine supplementation improved clinical status without changing oxidative stress and lipid profile in women with knee osteoarthritis

Aida Malek Mahdavi[a], *Reza Mahdavi*[b,*], *Sousan Kolahi*[c], *Maryam Zemestani*[a], *Amir-Mansour Vatankhah*[d]

[a] *Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran*
[b] *Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[c] *Connective Tissue Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*
[d] *Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran*

ARTICLE INFO

Article history:
Received 7 March 2015
Revised 14 May 2015
Accepted 8 June 2015

Keywords:
L-Carnitine
Lipid profile
Oxidative stress
Clinical status
Knee osteoarthritis
Women

ABSTRACT

Considering the pathologic importance of oxidative stress and altered lipid metabolism in osteoarthritis (OA), this study aimed to investigate the effect of L-carnitine supplementation on oxidative stress, lipid profile, and clinical status in women with knee OA. We hypothesized that L-carnitine would improve clinical status by modulating serum oxidative stress and lipid profile. In this randomized double-blind, placebo-controlled trial, 72 overweight or obese women with mild to moderate knee OA were randomly allocated into 2 groups to receive 750 mg/d L-carnitine or placebo for 8 weeks. Dietary intake was evaluated using 24-hour recall for 3 days. Serum malondialdehyde (MDA), total antioxidant capacity (TAC) and lipid profile, visual analog scale for pain intensity, and patient global assessment of severity of disease were assessed before and after supplementation. Only 69 patients (33 in the L-carnitine group and 36 in the placebo group) completed the study. L-Carnitine supplementation resulted in significant reductions in serum MDA (2.46 ± 1.13 vs 2.16 ± 0.94 nmol/mL), total cholesterol (216.09 ± 34.54 vs 206.12 ± 39.74 mg/dL), and low-density lipoprotein cholesterol (129.45 ± 28.69 vs 122.05 ± 32.76 mg/dL) levels compared with baseline ($P < .05$), whereas these parameters increased in the placebo group. Serum triglyceride, high-density lipoprotein cholesterol, and TAC levels did not change significantly in both groups ($P > .05$). No significant differences were observed in dietary intake, serum lipid profile, MDA, and TAC levels between groups after adjusting for baseline values and covariates ($P > .05$). There were significant intragroup and intergroup differences in pain intensity and patient global assessment of disease status after supplementation ($P < .05$). Collectively, L-carnitine improved clinical status without changing oxidative stress and lipid profile significantly in women with knee OA.

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfanate); ANCOVA, analysis of covariance; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; OA, osteoarthritis; TAC, total antioxidant capacity; TC, total cholesterol; TG, triglyceride; VAS, visual analog scale.

* *Corresponding author*. Nutrition Research Center, Faculty of Nutrition, Tabriz University of Medical Sciences, Golgasht St, Attar Neishabouri Ave, Tabriz, Iran. Tel.: +98 4133341113; fax: +98 4133355282.
E-mail address: mahdavirez@hotmail.com (R. Mahdavi).

http://dx.doi.org/10.1016/j.nutres.2015.06.003
0271-5317/

1. Introduction

Osteoarthritis (OA) is a common age-related disabling disease characterized by degradation and loss of articular cartilage as well as osteophyte formation [1]. It is the most common musculoskeletal disorder that can develop in any joint in the body, but knees are one of the most commonly affected joints because they are the primary weight-bearing joints of the body [2]. The prevalence of this debilitating disease is higher in women than in men [3].

The mechanisms responsible for development and progression of OA are very complex and poorly understood. It has been suggested that major mechanism underlying pathogenesis of OA is continuous oxidative stress to cells and matrix. In fact, when reactive oxygen species production exceeds the antioxidant defense capacities of chondrocytes, oxidative stress occurs leading to collagen, proteoglycans, and hyaluronan oxidation and finally to structural and functional cartilage damage [4]. Degradation products and oxidized molecules may contribute to synovial inflammation and form a vicious circle, consisting of new reactive oxygen species formation and further degradation products. Furthermore, several studies suggest that lipids might be involved in OA pathogenesis. In an early study, a positive association was observed between high serum cholesterol levels and hand OA in women [5]. In the Chingford [6], Ulm [7] and Saudi Arabian [8] studies, an association between high serum cholesterol levels and radiologic evidence of OA has been reported, which further supports the hypothesis that serum cholesterol is a systemic risk factor for OA. It has been reported that altered lipid metabolism is associated with the development of new bone marrow lesions, which are related to the progression of cartilage defects [9] and loss of it [10–12]. In addition, it has been proposed that active inflammatory environment associated with OA may result from disordered lipid metabolism, possibly through inflammatory pathways [13].

Common therapies for OA include lifestyle modifications and pharmacologic agents. Conventional drug treatments include analgesics, anti-inflammatory agents, hyaluronic acid, and intra-articular glucocorticoids [14]. To avoid the cardiac risks and gastrointestinal complications associated with traditional OA treatments particularly with long-term use [15,16], there has been a focus on complementary and alternative medicines recently, especially nutritional supplements. Considering the pathologic importance of oxidative stress and altered lipid metabolism in OA, it seems that administration of dietary supplements, which decrease oxidative stress and have beneficial effects on lipid levels, would be useful in managing OA. L-Carnitine is a dietary supplement that plays an important role in shuttling the long-chain fatty acids across the inner mitochondrial membrane for β-oxidation and ATP production [17] and prevents the accumulation of end-products of lipid peroxidation due to its antioxidant effects [18]. According to previous studies, levels of malondialdehyde (MDA) decreased significantly after L-carnitine supplementation in patients with type 2 diabetes [19], coronary artery disease [20], and hemodialysis [21,22]. Furthermore, Malaguarnera et al [19] reported that L-carnitine supplementation in patients with type 2 diabetes and nonalcoholic steatohepatitis [23] led to a significant decrease in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels. Recently, L-carnitine was reported to be effective in management of arthritis [24–26]. According to Bianchi et al [25], acetyl-L-carnitine was able to attenuate pain in OA rat knee. There has been only one study that investigated the effect of a food supplement sachet containing L-carnitine fumarate (345 mg) in patients with knee OA, which concluded that visual analog scale (VAS) score decreased significantly after the supplementation [26].

Considering the antioxidant and lipid-lowering properties of L-carnitine and because there was no study investigated these properties in OA patients as well as the scarcity of data regarding the effects of L-carnitine on clinical status, we hypothesized that L-carnitine supplementation would improve clinical status by modulating serum oxidative stress and lipid profile. To test the hypothesis, this study was designed to evaluate the effects of L-carnitine supplementation on serum oxidative stress, lipid profile, and clinical status in women with knee OA.

2. Methods and materials

2.1. Study participants

The study was conducted from November 2013 to November 2014. The sample size was calculated based on information obtained from studies by Farid et al [2] and Geraci et al [26] on the intensity of knee pain. Considering a confidence level of 95% and power of 80%, the sample was determined with at least 30 cases in each group. The sample size was increased to 36 cases in each group for a possible dropout of 20%. Seventy-two volunteer women 40 to 60 years of age with the diagnosis of mild to moderate bilateral primary knee OA according to the American College of Rheumatology criteria [27,28] and a body mass index (BMI) of 25 to 34.9 kg/m^2 were recruited from the rheumatology clinics of Tabriz University of Medical Sciences. The exclusion criteria were as follows: secondary OA (due to a known disorder); arthroscopy, surgery, or a joint injection of the target knee within the previous 6 months; history of knee joint replacement; any serious systematic disease, cardiovascular disease, diabetes mellitus, liver, renal and/or thyroid disorders, and any other chronic inflammatory disease; pregnancy and lactation; smoking; alcohol intake; consumption of lipid-lowering medications, omega-3-fatty acids (eg, fish oil), and antioxidant supplements; and use of nonsteroidal anti-inflammatory drugs 2 weeks prior to and during the intervention. Because the use of nonsteroidal anti-inflammatory drugs was not allowed during the trial, patients were permitted to use acetaminophen per day for relieving pain and symptoms if needed. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Iran) and registered on the Iranian Registry of Clinical Trials Web site (code: IRCT201311231197N17). All subjects were made aware of the content of the study, and a written informed consent was obtained from each subject.

2.2. Study design

This study was a randomized, double-blind, placebo-controlled trial. The eligible participants were randomly allocated into the intervention and placebo groups based on random block procedure consisting of 4 subjects per block, which matched subjects to each block based on menopausal status, BMI and age, produced by Random Allocation Software, version 1.0 (M. Saghaei, Department of Anesthesia, Isfahan University of Medical Sciences, Isfahan, Iran) [29]. A computer-generated random sequence was kept in a remote secure location and administered by an independent third party who was not involved with the clinical conduct of study until all data were collected and verified. Patients and those involved in enrolling participants, administering interventions, and assessing outcomes were blind to group assignments. The experimental group (n = 36) received 750 mg L-carnitine tartrate per day divided into 3 equal doses of one 250-mg tablet after each meal for 8 weeks (L-carnitine; Karen Pharmaceutical & Nutrilife Co, Yazd, Iran). The control group (n = 36) received placebo according to the same regimen and for the same duration (placebo; Karen Pharmaceutical & Nutrilife Co). The placebo pills contained inactive ingredients with no therapeutic activity and had an identical appearance. The participants were asked to keep their usual dietary intake and physical activity during the study period. Patients were monitored weekly for any adverse effects of L-carnitine supplementation. A diagram of the study design is shown in Fig. 1.

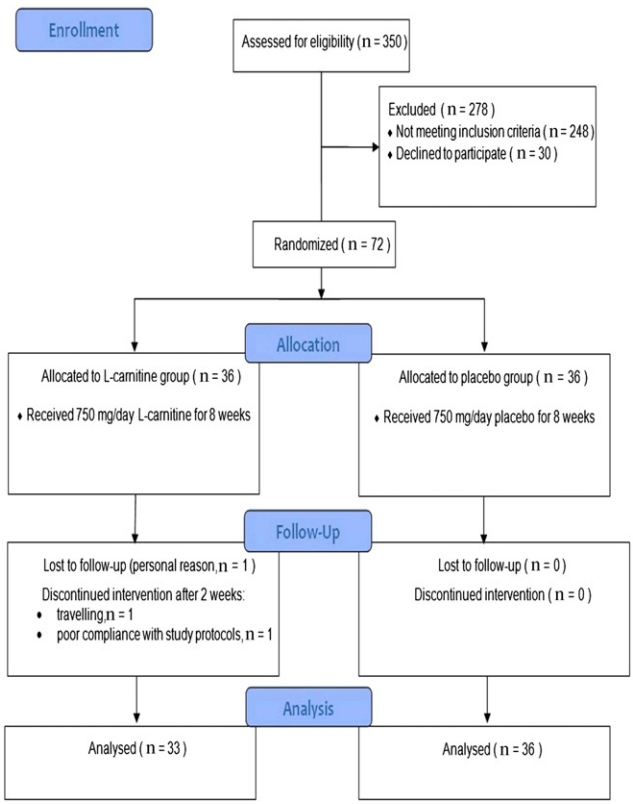

Fig. 1 – Study flow diagram.

2.3. Physical activity, anthropometry, and dietary intake assessments

At the onset of the study, all patients underwent routine physical examinations. A general questionnaire was completed for each subject, and physical activity levels of the patients were assessed using the short form of the International Physical Activity Questionnaire by an instructed interviewer [30]. The patients were classified as having high, moderate, or low physical activity levels according to the categorical scoring protocol of the short form of International Physical Activity Questionnaire [31]. At the beginning and at the end of the intervention period, body weight was measured to the nearest 0.5 kg using a Seca scale (Hamburg, Germany), with the patients being barefoot and wearing light clothing. Height was also measured using a mounted tape, with the participants' arms hanging freely by their sides and recorded to the nearest 0.5 cm. Body mass index was calculated by dividing weight (in kilograms) by the square of height (in meters) [32]. Information on food intake was collected by using a 24-hour recall method for 3 days (including 2 working days and 1 weekend) a week before and at the end of supplementation. Dietary intake of subjects was analyzed with the Nutritionist IV software program (First Databank Inc, Hearst Corp, San Bruno, CA, USA).

2.4. Blood sampling

At the beginning and at the end of the trial period, 5 mL of venous blood samples was collected after 12-hour overnight fasting. The serum samples were separated from whole blood by centrifugation at 3200 rpm for 10 minutes and were kept at –80°C until biochemical analysis.

2.5. Serum MDA

Serum MDA levels (used as a marker for oxidative stress) were determined through a reaction with thiobarbituric acid (TBA) as a TBA-reactive substance [33]. Briefly, serum samples were mixed with 3 mL of 1.0% phosphoric acid and 1.0 mL of 0.67% TBA, and then heated in a boiling water bath for 45 minutes. After cooling, 3 mL of *n*-butanol was added and the mixture was centrifuged at 3000 rpm for 10 minutes to separate into 2 layers. Thiobarbituric acid–reactive substance contents of the *n*-butanol layer were spectrophotometrically determined at 532 nm.

2.6. Serum total antioxidant capacity

Serum total antioxidant capacity (TAC) was measured using a Randox total antioxidant status kit (Crumlin, County Antrim, United Kingdom), in which 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfanate) (ABTS) is incubated with a peroxidase and H_2O_2 to produce the radical cation $ABTS^+$. This has a stable blue-green color, which is measured at 600 nm on an automatic analyzer (Abbott model Alcyon 300; Abbott Laboratories, Abbott Park, IL, USA). Antioxidants in the added sample cause suppression of this color production to a degree that is proportional to their concentration [34].

2.7. Serum lipid profile

Serum TC, TG, and high-density lipoprotein cholesterol (HDL-C) were measured using the standard enzymatic-colorimetric method by Bionik Diagnostic Kits (Tehran, Iran). Methods sensitivities were 1, 1, and 1 mg/dL, respectively. The reference range value for cholesterol, based on this method, was lower than 200 mg/dL, whereas top-edge range values were 200 to 240 mg/dL and high-level range values were higher than 240 mg/dL. The reference range value for TG was lower than 200 mg/dL, whereas top-edge range values were 200 to 400 mg/dL and high level range values were higher than 400 mg/dL. The reference range value for HDL-C was more than 35 mg/dL. Low-density lipoprotein cholesterol concentration was determined using the Friedewald formula [23]: LDL-C = TC − (HDL-C + TG/5).

2.8. Clinical status assessment

A VAS assessment of pain was also included. With this assessment, a line of 100 mm is drawn to measure the individuals' pain status, with 0 representing no pain and 100 being unbearable pain. Patients marked the relevant amount of pain experienced on this line, and the value was noted by the investigator, in millimeters [35]. Based on the distribution of pain VAS scores in patients who described their pain intensity as none, mild, moderate, or severe, the following cut points on the pain VAS were recommended: no pain (0-4 mm), mild pain (5-44 mm), moderate pain (45-74 mm), and severe pain (75-100 mm) [36]. In addition, the patient global assessment of the severity of knee OA, measured on a 0- to 100-mm VAS, was evaluated, where 0 equals no symptoms and higher scores indicate a lower response to therapy (or more severe the disease) [37]. Similar to pain status, patients marked the relevant severity of disease experienced on this line and the value was noted by the investigator, in millimeters [35].

2.9. Statistical analyses

Statistical analysis was performed using SPSS version 16.0 software (SPSS, Inc, Chicago, IL, USA). Normality of variables distribution was evaluated using the Kolmogorov-Smirnov test. Variables not normally distributed were analyzed using a nonparametric test. Qualitative and normally distributed quantitative variables were displayed as numbers (percentages) and means ± SD, respectively. Nonnormally distributed quantitative variables were presented as median (interquartile range). Demographic variables were analyzed using a χ^2 or independent-sample *t* test, as appropriate. The differences between variables before and after intervention were compared by paired *t* test or Wilcoxon signed rank test. Between-group comparisons were made by independent-sample *t* test or Mann-Whitney *U* test. Analysis of covariance (ANCOVA) was used to identify any differences between the 2 groups at the end of the study, adjusting for baseline values and covariates (duration of OA, changes of weight and calorie intake, and antioxidant micronutrients intake such as vitamins A, C, E, and minerals zinc and selenium). The Sign and Mann-Whitney *U* tests were used for intragroup and intergroup comparisons of the qualitative data, respectively. $P < .05$ was considered statistically significant.

3. Results

3.1. Participant demographics

From a total of 72 subjects who met the inclusion criteria and entered the study, 3 subjects in the L-carnitine group were withdrawn due to discontinuing intervention (traveling and poor compliance with study protocols) and lost to follow-up (personal reason). Therefore, the data were reported for 69 patients (33 in the L-carnitine group and 36 in the placebo group; Fig. 1). Participants did not report any adverse effects or symptoms with the L-carnitine consumption or placebo during the study, which confirmed the safety of L-carnitine in the present study as well as previous trials. Overall, the use of acetaminophen was low and very similar in both treatment groups throughout the study (21.2% in the L-carnitine group and 22.2% in the placebo group). There were no significant differences between the groups for acetaminophen use ($P > .05$). The means ± SD age and BMI of the participants were 52.05 ± 6.13 years and 32.02 ± 3.12 kg/m^2, respectively. Thirty-eight female participants (55.1%) had reached menopause. As presented in Table 1, there were no significant differences in demographic characteristics, duration of disease, or in physical activity level between the study groups at baseline.

3.2. Anthropometric measures and daily dietary intake

Table 2 presents the anthropometric measures and daily dietary intake details of participants throughout the study. No significant changes were seen between and within groups in weight and BMI of subjects after 8 weeks of intervention ($P > .05$). In comparison with baseline, total energy, carbohydrate, and selenium intake decreased significantly in the L-carnitine group by 7.88%, 9.14%, and 22.33%, respectively ($P < .05$).

Table 1 – Baseline characteristics of study subjects

Variable	L-Carnitine group (n = 33)	Placebo group (n = 36)	P [a]
Age (y)	51.63 ± 5.69	52.44 ± 6.56	.588
Weight (kg)	77.42 ± 9.62	78.27 ± 10.18	.722
Height (cm)	156.48 ± 6.01	155.22 ± 6.72	.415
BMI (kg/m^2)	31.57 ± 3.06	32.43 ± 3.16	.256
Duration of OA (y)	4.13 ± 3.83	5.83 ± 5.92	.167
Menopause status, n (%)			.933
Not menopause	15 (45.5)	16 (44.4)	
Menopause	18 (54.5)	20 (55.6)	
Physical activity level, n (%)			.575
Low	19 (57.6)	19 (52.8)	
Moderate	13 (39.4)	14 (38.9)	
High	1 (3.0)	3 (8.3)	

Values are means ± SD, unless otherwise indicated.
$P < .05$ was considered significant.
[a] *P* values indicate comparison between groups at baseline (independent-sample *t* test, χ^2, or Mann-Whitney *U* test, as appropriate).

Table 2 – Anthropometric measures and dietary intake of subjects at baseline and after 8 weeks

Variable		L-Carnitine group (n = 33)	Placebo group (n = 36)	P [a]
Weight (kg)	Baseline	77.42 ± 9.62	78.27 ± 10.18	.722
	After 8 wk	76.86 ± 9.97	78.16 ± 10.80	.357
	P [b]	.069	.747	
BMI (kg/m^2)	Baseline	31.57 ± 3.06	32.43 ± 3.16	.256
	After 8 wk	31.34 ± 3.24	32.37 ± 3.32	.461
	P [b]	.078	.662	
Energy (kcal/d)	Baseline	1883.27 ± 381.23	1845.64 ± 418.90	.698
	After 8 wk	1734.69 ± 442.34	1763.03 ± 416.91	.566
	P [b]	.012	.281	
Carbohydrate (g/d)	Baseline	300.92 ± 77.29	293.24 ± 76.13	.679
	After 8 wk	273.39 ± 80.03	277.58 ± 78.62	.641
	P [b]	.025	.305	
Protein (g/d)	Baseline	64.04 ± 12.95	63.66 ± 13.45	.907
	After 8 wk	63.60 ± 16.16	60.43 ± 15.75	.413
	P [b]	.885	.265	
Total fat (g/d)	Baseline	50.58 ± 16.09	50.04 ± 14.09	.881
	After 8 wk	45.98 ± 15.18	49.39 ± 17.55	.294
	P [b]	.095	.815	
Vitamin A (RE/d)	Baseline	1073.64 ± 813.38	905.39 ± 564.82	.319
	After 8 wk	944.77 ± 778.61	920.85 ± 537.36	.801
	P [b]	.337	.902	
Vitamin E (mg/d)	Baseline	2.89 (2.31-5.33)	2.12 (1.61-3.22)	.017
	After 8 wk	2.55 (1.53-5.44)	2.38 (1.94-3.80)	.676
	P [c]	.586	.376	
Vitamin C (mg/d)	Baseline	120.24 ± 76.44	98.37 ± 47.13	.163
	After 8 wk	112.85 ± 71.10	113.97 ± 89.47	.615
	P [b]	.648	.254	
Zinc (mg/d)	Baseline	5.86 ± 1.83	5.99 ± 1.91	.781
	After 8 wk	6.08 ± 2.41	5.75 ± 2.02	.427
	P [b]	.631	.446	
Selenium (mg/d)	Baseline	0.08 ± 0.02	0.06 ± 0.02	.012
	After 8 wk	0.06 ± 0.02	0.06 ± 0.02	.915
	P [b]	.013	.771	

Vitamin E is reported as median (interquartile range), and other values are reported as means ± SD.
$P < .05$ was considered significant.
[a] P values indicate comparison between groups (independent-sample t test and/or Mann-Whitney U test for normally and nonnormally distributed measures, respectively, at baseline or ANCOVA test, adjusted for baseline values, after 8 weeks).
[b] P values indicate comparison within groups (paired t test).
[c] P values indicate comparison within groups (Wilcoxon signed rank test).

Except for selenium and vitamin E, there were no significant differences between the 2 groups for energy, macronutrients, and other micronutrients intake at baseline ($P > .05$). At the end of the study, results of ANCOVA test did not show statistically significant differences between the 2 studied groups in energy, macronutrients, and micronutrients intake, adjusted for baseline values ($P > .05$).

3.3. *Serum oxidative stress parameters*

As illustrated in Table 3, the independent-sample t test results did not show significant differences between the 2 groups in terms of serum MDA and TAC levels at baseline ($P > .05$). Serum MDA level decreased significantly in the L-carnitine group by 12.30% ($P < .05$), whereas it increased insignificantly by 4.73% in the placebo group ($P > .05$) after the experimental period. The mean serum TAC levels did not change significantly in both groups after the study. Results of ANCOVA test also did not show statistically significant differences between the 2 studied groups in serum MDA and TAC levels at the end of the study ($P > .05$), adjusted for baseline values and covariates (Table 3).

3.4. *Serum lipid levels*

As shown in Table 4, the independent-sample t test results revealed no significant differences between the 2 groups in terms of serum lipid levels at baseline ($P > .05$). Serum levels of TC and LDL-C decreased significantly in the L-carnitine group by 4.61% and 5.71%, respectively, whereas these parameters increased insignificantly by 1.87% and 2.13%, respectively, in the placebo group, at the end of the study compared with baseline values (Table 4). Levels of serum TG and HDL-C did not change significantly in both groups at the end of the intervention. At the end of the study, results of ANCOVA test did not show statistically significant differences between the 2 studied groups in serum TG, TC, HDL-C, and LDL-C levels, adjusted for baseline values and covariates ($P > .05$).

Table 3 – Serum oxidative stress status of subjects at baseline and after 8 weeks

Variable		L-Carnitine group (n = 33)	Placebo group (n = 36)	P [a]
MDA (nmol/mL)	Baseline	2.46 ± 1.13	2.26 ± 0.79	.400
	After 8 wk	2.16 ± 0.94	2.37 ± 0.97	.338
	P [b]	.045	.483	
TAC (mmol/L)	Baseline	1.77 ± 0.32	1.73 ± 0.22	.545
	After 8 wk	1.71 ± 0.24	1.73 ± 0.28	.784
	P [b]	.098	.826	

Values are means ± SD.
$P < .05$ was considered significant.
[a] P values indicate comparison between groups (independent-sample t test at baseline and ANCOVA test, adjusted for baseline values, duration of OA, weight, calorie intake, and antioxidant micronutrients intake changes, after 8 weeks).
[b] P values indicate comparison within groups (paired t test).

3.5. *Clinical status*

Table 5 shows the distribution of pain intensity in the L-carnitine and placebo groups before and after the study. At baseline, there were no significant differences in pain intensity between the study groups ($P > .05$). At the end of the study, significant differences in pain intensity were observed between the 2 groups ($P < .05$). Results of Sign test also showed significant intragroup changes in pain intensity in the L-carnitine ($P < .001$) and placebo ($P = .012$) groups. Fig. 2 depicts the patient global assessment of the severity of knee OA before and after intervention in the L-carnitine and placebo groups. At baseline, there were no significant differences between the 2 groups for mean patient global assessment of the severity of knee OA ($P = .536$). Patient global assessment of the severity of knee OA decreased significantly by 46.48% in the L-carnitine group ($P < .001$) and by 8.58% in the placebo group ($P = .012$) after supplementation. At the end of the study, ANCOVA test revealed a significant difference between the 2 groups for mean patient global assessment of the severity of knee OA ($P < .001$), adjusted for baseline values, weight and calorie intake changes, and duration of OA.

4. Discussion

It has been proposed that lipid peroxidation products, that is, MDA levels, as an indicator of oxidative stress, increase significantly in patients with OA [38,39]. Moreover, it has been reported that plasma TAC levels are significantly lower in patients with OA compared with healthy subjects [40]. Therefore, treatment with antioxidants may be helpful as a secondary therapy to prevent further oxidative damage and deterioration of the musculoskeletal tissues in OA. L-Carnitine is a dietary supplement with known antioxidant properties and has been reported to have beneficial effects in common chronic diseases. Previous studies have shown that L-carnitine supplementation decreases MDA concentrations significantly in healthy women [41], patients with type 2 diabetic [19], patients with coronary artery disease [20], patients undergoing hemodialysis [21,22]. Lee et al [20] reported that L-carnitine supplementation increased levels of antioxidant enzymes activities (eg, catalase, superoxide dismutase, glutathione peroxidase) significantly in patients with coronary artery disease. In addition, Cao et al [42] demonstrated that carnitine administration led to a gradual increase in plasma concentrations of TAC in healthy subjects. These observations support the antioxidant role of carnitine, which is most likely due to stabilization of various membranes, including the mitochondria [43], increased levels of different antioxidant enzymes [44], inhibition of microsomal peroxidation [45,46], and prevention of fatty acid membrane peroxidation [47]. In the present study,

Table 4 – Serum lipid profile of subjects at baseline and after 8 weeks

Variable		L-Carnitine group (n = 33)	Placebo group (n = 36)	P [a]
TG (mg/dL)	Baseline	167.00 ± 94.39	140.39 ± 62.77	.169
	After 8 wk	164.45 ± 86.32	139.36 ± 67.67	.465
	P [b]	.812	.890	
TC (mg/dL)	Baseline	216.09 ± 34.54	202.53 ± 26.92	.072
	After 8 wk	206.12 ± 39.74	206.33 ± 40.78	.111
	P [b]	.015	.482	
HDL-C (mg/dL)	Baseline	53.24 ± 11.19	51.91 ± 11.76	.634
	After 8 wk	51.18 ± 10.82	53.30 ± 13.03	.070
	P [b]	.117	.268	
LDL-C (mg/dL)	Baseline	129.45 ± 28.69	122.53 ± 22.58	.268
	After 8 wk	122.05 ± 32.76	125.16 ± 34.39	.249
	P [b]	.041	.630	
TC/HDL-C	Baseline	4.16 ± 0.80	4.05 ± 0.89	.590
	After 8 wk	4.10 ± 0.77	4.04 ± 1.10	.872
	P [b]	.540	.914	

Values are means ± SD.
Abbreviation: TC/HDL-C, TC/HDL-C ratio.
$P < .05$ was considered significant.
[a] P values indicate comparison between groups (independent-sample t test at baseline and ANCOVA test, adjusted for baseline values, duration of OA, weight, and calorie intake changes, after 8 weeks).
[b] P values indicate comparison within groups (paired t test).

Table 5 – Pain intensity of subjects before and after intervention

Variable		L-Carnitine group (n = 33)				Placebo group (n = 36)				P [a]
		No pain	Mild	Moderate	Severe	No pain	Mild	Moderate	Severe	
Pain intensity	Before	0 (0.0)	10 (30.3)	19 (57.6)	4 (12.1)	0 (0.0)	15 (41.7)	15 (41.7)	6 (16.7)	.593
	After	2 (6.1)	26 (78.8)	5 (15.2)	0 (0.0)	0 (0.0)	23 (63.9)	10 (27.8)	3 (8.3)	.019
	P [b]	<.001				.012				

Pain intensity was assessed using a 0- to 100-mm VAS scale, with 0 representing no pain and 100 being unbearable pain. Based on distribution of pain VAS scores in patients who described their pain intensity as none, mild, moderate, or severe, the following cut points on the pain VAS were recommended: no pain (0-4 mm), mild pain (5-44 mm), moderate pain (45-74 mm), and severe pain (75-100 mm).
Values are number (percentage) of subjects per group.
$P < .05$ was considered significant.
[a] P values indicate comparison between groups (Mann-Whitney U test).
[b] P values indicate comparison within groups (Sign test).

a significant reduction in serum MDA levels occurred in the L-carnitine group from baseline to eighth week; however, L-carnitine supplementation did not lead to a significant decrease in serum MDA levels compared with the placebo group. In addition, we did not observe any significant differences in serum TAC levels within or between groups. This discrepancy between our findings with previous studies might be due to differences in studied population, baseline MDA and TAC status, and dosage and duration of L-carnitine supplementation.

Moreover, our results indicated significant reductions in serum TC and LDL-C levels in the L-carnitine group compared with baseline; however, no significant differences were observed in serum lipid levels between the L-carnitine and placebo groups after supplementation. Our findings were in line with the results of previous studies in obese women [48] and in patients with type 2 diabetes [49]. The lack of difference in serum lipid profile between the 2 groups might be due to the fact that participants' lipid profile was within the reference range, similar to the normal-weight participants. These results differ from other authors' findings in patients with type 2 diabetes [19] and patients with nonalcoholic steatohepatitis [23], in which the studied patients were hyperlipidemic. This inconsistency between results of different studies might be attributable to differences in baseline lipid profile status, physiological circumstances (eg, different types of disease), and dosage and duration of L-carnitine administration. It has been proposed that L-carnitine supplementation decreases serum LDL-C levels via different mechanisms such as reducing level of fatty acid inflow for LDL-C, up-regulation of LDL receptors, and decreasing TC, apolipoprotein B100, and TG levels, as well as the generation of subnormal very LDLs [50,51].

Clinically, OA is characterized by joint pain, tenderness, limitation of movement, crepitus, and occasional effusion [52]. Pain is the most prominent and disabling symptom of OA which is associated with functional outcomes and reduced quality of life [53]. Currently, there is no apparent cure for OA. Therefore, the goals of treatments are to alleviate the pain and symptoms of the disease and, if possible, to slow its progression. In present study, we noted significant within-group reductions in pain intensity and patient global assessment of severity of knee OA at week 8 compared with baseline among subjects given both L-carnitine and placebo; however, the magnitude of this reduction was higher in the L-carnitine group, which led to significant differences between the 2 groups at the end of the study. The real mechanism underlying this event is not clear, but because we found no significant differences in serum oxidative stress parameters and lipid profile between the L-carnitine and placebo groups, we supposed that further studies are needed to elucidate the exact mechanism of improving clinical status by L-carnitine in patients with knee OA. It has been reported that placebo could be effective in treatment of OA, especially for pain [54]. Because there were no significant differences between the groups for acetaminophen use during the study, the reduction of pain intensity in the placebo group is most likely due to the *placebo effect*. Our findings were in agreement with the only similar study carried out by Geraci et al [26], in which a food supplement sachet containing L-carnitine fumarate (345 mg) decreased pain intensity significantly in patients with knee OA after the intervention ($P < .05$).

The limitations of the present study included a short duration of the intervention and low dose of treatment. In addition, we did not evaluate serum L-carnitine levels of the

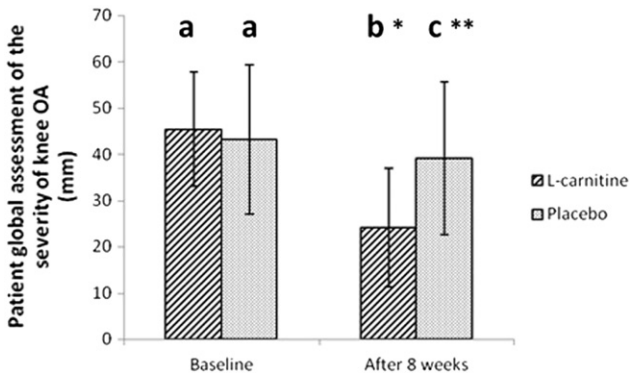

Fig. 2 – Patient global assessment of the severity of knee OA (100-mm VAS) in the treatment groups at baseline and after 8 weeks. Values are means ± SD (n = 33 in the L-carnitine group and n = 36 in the placebo group). The data were tested using independent-sample t test at baseline and ANCOVA test, adjusted for baseline values, duration of OA, weight, and calorie intake changes, after 8 weeks. Means that do not share a common letter indicate statistical difference at $P < .05$. The mean values were significantly different compared with baseline in both groups (paired t test): $^{*}P < .001$; $^{**}P < .05$.

patients. The strength of our study was monitoring patient status by weekly telephone conversations. Moreover, L-carnitine appeared to be well tolerated by participants and anecdotal reports indicated that the intervention was acceptable to them. In conclusion, L-carnitine improved clinical status without changing oxidative stress and lipid profile significantly in women with knee OA. Therefore, we reject the hypothesis that L-carnitine would improve clinical status in women with knee OA by modulating oxidative stress and lipid profile. Long-term studies with higher doses of L-carnitine and measuring other indicators of oxidative damage in hyperlipidemic patients with OA would help to further clarify the subject.

Conflict of interest

The authors have no conflicts of interest.

Acknowledgment

The results of this article are derived from the PhD thesis of Aida Malek Mahdavi (No. D/36) registered in the Tabriz University of Medical Sciences, Tabriz, Iran. The authors wish to thank the Nutrition Research Center of Tabriz University of Medical Sciences for financial support. They also thank all the patients for their participation in this research.

REFERENCES

[1] Salaffi F, De Angelis R, Stancati A, Grassi W, MArche Pain, Prevalence Investigation Group (MAPPING) Study. Health-related quality of life in multiple musculoskeletal conditions: a cross-sectional population based epidemiological study. II. The MAPPING study. Clin Exp Rheumatol 2005;23:829–39.
[2] Farid R, Rezaie yazdi Z, Mirfeizi Z, Hatef MR, Mirheidari M, Mansouri H, et al. Oral intake of purple passion fruit peel extract reduces pain and stiffness and improves physical function in adult patients with knee osteoarthritis. Nutr Res 2010;30:601–6.
[3] Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. Curr Opin Rheumatol 2001;13:447–51.
[4] Yudoh K, Van Trieu N, Nakamura H, Hongo-Masuko K, Kato T, Nishioka K. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and down-regulation of chondrocyte function. Arthritis Res Ther 2005;7:380–91.
[5] Kellgren JH. Osteoarthrosis in patients and populations. Br Med J 1961;2:1–6.
[6] Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford study. J Rheumatol 1995;22:1118–23.
[7] Sturmer T, Sun Y, Sauerland S, Zeissig I, Gunther KP, Puhl W, et al. Serum cholesterol and osteoarthritis. The baseline examination of the Ulm Osteoarthritis study. J Rheumatol 1998;25:1827–32.
[8] Al-Arfaj AS. Radiographic osteoarthritis and serum cholesterol. Saudi Med J 2003;24:745–7.
[9] Hanna FS, Bell RJ, Cicuttini FM, Davison SL, Wluka AE, Davis SR. High sensitivity C-reactive protein is associated with lower tibial cartilage volume but not lower patella cartilage volume in healthy women at midlife. Arthritis Res Ther 2008;10:R27–33.
[10] Garnero P, Peterfy C, Zaim S, Schoenharting M. Bone marrow abnormalities on magnetic resonance imaging are associated with type II collagen degradation in knee osteoarthritis. Arthritis Rheum 2005;52:2822–9.
[11] Phan CM, Link TM, Blumenkrantz G, Dunn TC, Ries MD, Steinbach LS, et al. MR imaging findings in the follow up of patients with different stages of knee osteoarthritis and the correlation with clinical symptoms. Eur Radiol 2006;16:608–18.
[12] Hunter DJ, Zhang Y, Niu J. Increase in bone marrow lesions associated with cartilage loss: a longitudinal magnetic resonance imaging study of knee osteoarthritis. Arthritis Rheum 2006;54:1529–35.
[13] Karvonen-Gutierrez CA. Knee osteoarthritis: intersections of obesity, inflammation, and metabolic dysfunction. PhD dissertation The University of Michigan; 2012.
[14] Bijlsma JW, Knahr K. Strategies for the prevention and management of osteoarthritis of the hip and knee. Best Pract Res Clin Rheumatol 2007;21:59–76.
[15] Phillips CR, Brasington RD. Osteoarthritis treatment update: are NSAIDs still in the picture? Musculoskelet Med 2010;27:65–71.
[16] Berenbaum F. New horizons and perspectives in the treatment of osteoarthritis. Arthritis Res Ther 2008;10:S1–7.
[17] Chapela SP, Kriguer N, Ferna´ndez EH, Stella CA. Involvement of L-carnitine in cellular metabolism: beyond acyl-CoA transport. Mini Rev Med Chem 2009;9:1518–26.
[18] Dokmeci D, Akpolat M, Aydogdu N, Doganay L, Turan FN. L-Carnitine inhibits ethanol-induced gastric mucosal injury in rats. Pharmacol Rep 2005;57:481–8.
[19] Malaguarnera M, Vacante M, Avitabile T, Malaguarnera M, Cammalleri L, Motta M. L-Carnitine supplementation reduces oxidized LDL cholesterol in patients with diabetes. Am J Clin Nutr 2009;89:71–6.
[20] Lee BJ, Lin JS, Lin YC, Lin PT. Effects of L-carnitine supplementation on oxidative stress and antioxidant enzymes activities in patients with coronary artery disease: a randomized, placebo-controlled trial. Nutr J 2014;13:79–85.
[21] Fatouros IG, Douroudos I, Panagoutsos S, Pasadakis P, Nikolaidis MG, Chatzinikolaou A, et al. Effects of L-carnitine on oxidative stress responses in patients with renal disease. Med Sci Sports Exerc 2010;42:1809–18.
[22] Mitwalli AH, Al-Wakeel JS, Alam A, Tarif N, Abu-Aisha H, Rashed M, et al. L-Carnitine supplementation in hemodialysis patients. Saudi J Kidney Dis Transpl 2005;16:17–22.
[23] Malaguarnera M, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, et al. L-Carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. Am J Gastroenterol 2010;105:1338–45.
[24] Stoppoloni D, Politi L, DallaVedova P, Messano M, Koverech A, Scandurra R, et al. L-Carnitine enhances extracellular matrix synthesis in human primary chondrocytes. Rheumatol Int 2013;33:2399–403.
[25] Bianchi E, Mannelli LDC, Menicacci C, Lorenzoni P, Aglianò M, Ghelardini C. Prophylactic role of acetyl-L-carnitine on knee lesions and associated pain in a rat model of osteoarthritis. Life Sci 2014;106:32–9.
[26] Geraci A, Zatta D, Strazzabosco C, Tomasello G, Alongi G, Genovese M, et al. The clinical effectiveness of glucosamine sulfate, chondroitin sulfate, hydrolyzed collagen type II, hydrolized hyaluronic acid and L-carnitine supplement in patients with osteoarthritis of the knee: a multicenter

randomized double blind controlled clinical trial. Minerva Ortopedica Traumatol 2012;63:9–17.
[27] Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986;29:1039–49.
[28] Massicotte F. Epidemiology of osteoarthritis. In: Martel-Pelletier J, Pelletier JP, editors. Understanding osteoarthritis from bench to bedside. Kerala-India: Research Signpost; 2011. p. 1–26.
[29] Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol 2004;4:1–6.
[30] Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International Physical Activity Questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 2003;35:1381–95.
[31] IPAQ research committee. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ)—short and long forms (November 2005); 2006.
[32] Hammond KA, Litchford MD. Clinical: inflammation, physical, and functional assessment. In: Mahan LK, Escott-Stump S, editors. Krause's food & nutrition therapy. Philadelphia – Pennsylvania: Saunders; 2012. p. 163–77.
[33] Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1978;86:271–8.
[34] Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application for monitoring the antioxidant status in premature neonates. Clin Sci 1993;84:407–12.
[35] Mehta K, Gala J, Bhasale S, Naik S, Modak M, Thakur H, et al. Comparison of glucosamine sulfate and a polyherbal supplement for the relief of osteoarthritis of the knee: a randomized controlled trial. BMC Complement Altern Med 2007;7:34–53.
[36] Jensen MP, Chen C, Brugger AM. Interpretation of visual analog scale ratings and change scores: a reanalysis of two clinical trials of postoperative pain. J Pain 2003;4:407–14.
[37] Snijders GF, van den Ende CHM, van Riel PLCM, van den Hoogen FHJ, den Broeder Alfons A. The effects of doxycycline on reducing symptoms in knee osteoarthritis: results from a triple-blinded randomised controlled trial. Ann Rheum Dis 2011;70:1191–6.
[38] Surapaneni KM, Venkataramana G. Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. Indian J Med Sci 2007;61:9–14.
[39] Gordon LA, Morrison EY, McGrowder DA, Young R, Fraser YT, Zamora EM, et al. Effect of exercise therapy on lipid profile and oxidative stress indicators in patients with type 2 diabetes. BMC Complement Altern Med 2008;8:21–30.
[40] Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatol Int 2007;27:339–44.
[41] Sachan DS, Hongu N, Johnsen M. Decreasing oxidative stress with choline and carnitine in women. J Am Coll Nutr 2005;24:172–6.
[42] Cao Y, Qu H, Li P, Wang C, Wang L, Han Z. Single dose administration of L-carnitine improves antioxidant activities in healthy subjects. Tohoku J Exp Med 2011;224:209–13.
[43] Binienda ZK. Neuroprotective effects of L-carnitine in induced mitochondrial dysfunction. Ann N Y Acad Sci 2003;993:289–95.
[44] Andrieu-Abadie N, Jaffrezou JP, Hatem S, Laurent G, Levade T, Mercadier JJ. L-Carnitine prevents doxorubicin-induced apoptosis of cardiac myocytes: role of inhibition of ceramide generation. FASEB J 1999;13:1501–10.
[45] Sushamakumari S, Jayadeep A, Kumar JS, Menon VP. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. Indian J Exp Biol 1989;27:134–7.
[46] Yasui F, Matsugo S, Ishibashi M, Kajita T, Ezashi Y, Oomura Y, et al. Effects of chronic acetyl-L-carnitine treatment on brain lipid hydroperoxide level and passive avoidance learning in senescence-accelerated mice. Neurosci Lett 2002;334:177–80.
[47] Kumaran S, Deepak B, Naveen B, Panneerselvam C. Effects of levocarnitine on mitochondrial antioxidant systems and oxidative stress in aged rats. Drugs R D 2003;4:141–7.
[48] Alshammari N. The effect of L-carnitine and physical activity on adipocytokines and lipid profile in obese women. World J Sport Sci 2011;4:21–3.
[49] Shakerhosseini R, Rahbar A, Saadat N, Pordal AH, Taleban FA, Golestan B. The effect of L-carnitine supplement on lipidemic and glycemic profile in patients with type II diabetes mellitus. Iran J Endocrinol Metab 2006;7:157–65.
[50] Hoppel C. The role of carnitine in normal and altered fatty acid metabolism. Am J Kidney Dis 2003;41:S4–S12.
[51] Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, et al. Small, dense low density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95:69–75.
[52] Seki T, Hasegawa Y, Yamaguchi J, Kanoh T, Ishiguro N, Tsuboi M, et al. Association of serum carotenoids, retinol, and tocopherols with radiographic knee osteoarthritis: possible risk factors in rural Japanese inhabitants. J Orthop Sci 2010;15:477–84.
[53] Hunter DJ, McDougall JJ, Keefe FJ. The symptoms of osteoarthritis and the genesis of pain. Rheum Dis Clin North Am 2008;34:623–43.
[54] Abhishek A, Doherty M. Mechanisms of the placebo response in pain in osteoarthritis. Osteoarthritis Cartilage 2013;21:1229–35.