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ORIGINAL ARTICLE

L-Carnitine supplementation reduces biomarkers of inflammatory and oxidative stress in patients with coronary artery disease: a randomised controlled trial

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ABSTRACT

Objective: L-Carnitine has been suggested as a potential nutrient that alleviates the oxidative and inflammatory damages of coronary artery disease (CAD), but the results of the previous studies of the importance of this supplementation remains unclear. This study attempts to evaluate the effects of L-carnitine (LC) supplementation on oxidative stress and inflammatory biomarkers in patients with CAD.

Methods: A double-blind, randomised, placebo-trial was conducted on 75 CAD subjects. Patients were randomly assigned to receive LC (1000 mg/day) or placebo capsules over 3 months. Sera high-sensitivity C-reactive protein (hs-CRP), myeloperoxidase (MPO), nitrotyrosine (NT) and total antioxidant capacity (TAC) were assayed.

Results: A significant increase in serum TAC and a significant decrease in MPO, NT, and hs-CRP levels were detected following 12 weeks of LC supplementation, compared to the placebo.

Conclusions: These results suggest that LC supplementation may exert beneficial effect on cardiovascular health through attenuate oxidative and inflammatory markers in CAD patients.

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KEYWORDS
L-Carnitine; coronary artery disease; oxidative stress; inflammatory; cardiovascular disease

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, a significant portion of which can be attributed to ischaemic heart disease, often as a result of underlying coronary artery disease (CAD) due to atherosclerosis (Glovaci *et al.* 2019, Sarrafzadegan and Mohammadifard 2019). It has been shown that reactive oxygen species (ROS) are dramatically increased in the intact canine myocardium following ischaemic occlusion (Zuluaga *et al.* 2018). This result has recently been confirmed in a similar study (Granger and Kvietys 2015). Inflammation, disturbed blood flow and arterial wall remodelling are the main characteristics of atherosclerosis, and excessive ROS production has a vital role in all these features (He and Zuo 2015).

Some evidence suggests that acute and chronic production of ROS in pathophysiologic conditions is essential for the progression of cardiovascular disease and the role of oxidative stress in the pathophysiology of cardiovascular disease is well established (de Freitas Brito *et al.* 2018). Studies have shown that ROS mediates several signalling pathways leading to vascular inflammation in atherosclerosis (de Freitas Brito *et al.* 2018; Figure 1). Atherosclerosis and coronary artery disease are more likely to be caused by inflammation and less likely to be due to the simple accumulation of lipids in the vessels (Granger and Kvietys 2015). Atherosclerosis is now considered to be a chronic inflammatory disease, and ROS are the possible stimuli to the inflammatory process (Hansson 2005). Studies have shown that pharmaceutical approaches for the treatment of CVD have limited efficiency (He and Zuo 2015). Considering the role of oxidative and inflammatory processes in the pathogenesis of heart disease, the use of antioxidant and anti-inflammatory compounds may be effective in preventing the progression and worsening of cardiovascular diseases.

L-Carnitine (LC, β-hydroxy-γ-trimethylaminobutyric acid) is a natural constituent of human cells (Johri *et al.* 2014). LC acts as an intermediary in the transfer of long-chain fatty acids into the mitochondria; therefore, it facilitates the mitochondrial β-oxidation cycle and energy production (Ribas *et al.* 2014). In addition to the important role of LC in fat metabolism, it has been shown that L-carnitine can act as an antioxidant (scavenger of free radicals) and anti-inflammatory agent which protects tissues from damages caused by ROS (Moeinian *et al.* 2013, Lee *et al.* 2016). Since carnitine levels of myocardium decrease rapidly in ischaemic conditions, it has been seen that consumption of exogenous carnitine in form of supplementation can recharge the evacuated levels of myocardium carnitine (Wang *et al.* 2018). The heart muscle is one of the organs which are not able to synthesise carnitine. Therefore, it is most affected by reducing the

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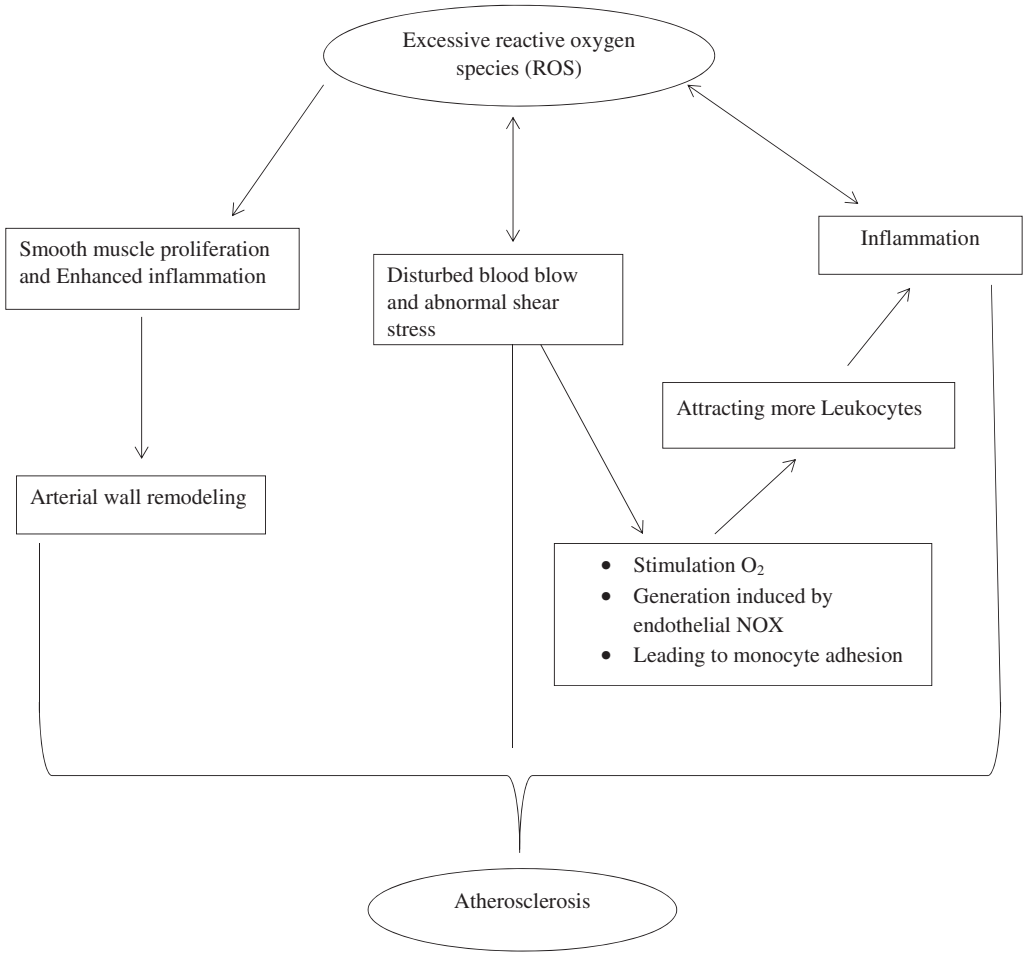

Figure 1. The role of ROS in formation of atherosclerotic plaques.

concentration of carnitine (Flanagan *et al.* 2010). This condition is a challenge for ischaemic heart tissue in energy supply.

Therefore, considering the anti-inflammatory and antioxidant effects of LC (Alvarez de Sotomayor *et al.* 2007, Volek *et al.* 2008) as well as the paucity of clinical trials on its therapeutic role in patients with CAD, the current study evaluated the effects of LC supplementation on inflammation and oxidative stress in patients with CAD.

Materials and methods

Subjects and study design

This study was a double-blind, randomised, placebo-controlled trial conducted on patients with coronary artery disease in Kermanshah, Iran, 2017. The study population consisted of all patients referred to the Angiography unit of Imam Ali Hospital in Kermanshah; but only 75 patients who met electrocardiographic and angiographic criteria were selected by a cardiologist to enter the study. Samples were selected using available sampling methods and sample size was calculated based on the study of Bor-Jen Lee *et al.* (2015).

Inclusion criteria included at least 50% stenosis in one or more major coronary arteries and lack of diseases (diabetes, liver or renal diseases, infectious diseases including hepatitis, inflammatory diseases and thyroid gland disorders). To minimise the influence of other cardiovascular risk factors, patients should not intake medications and supplements such as L-carnitine, vitamins E and C supplements, thyroid hormone, warfarin, antiepileptic and chemotherapy drugs.

Exclusion criteria included lack of individual interest in continuing cooperation, lack of compliance with prescribed levels of supplementation of L-carnitine and changes in the dosage of used medications. Written informed consent was obtained from all patients before study entry.

Intervention

Patients were randomly allocated into two groups. The LC group was given a capsule containing 1000 mg LC every day, whereas the placebo group received a capsule containing soluble starch every day, both for 12 weeks.

Biochemical indices

At baseline, before supplementation and after 12 weeks of supplementation, 10 ml of venous blood was collected after an overnight fast ($\geq$12 h). Plasma was obtained from the blood samples using EDTA-coated tubes, which were centrifuged at 3000 *g* for 15 min at 4 °C. Immediately after

centrifugation, the plasma samples were frozen and stored at −80 °C until they were analysed.

The serum nitrotyrosine (NT) concentration was measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Hangzhou East Biopharm Co., Ltd., USA). The intra- and inter-assay coefficient of variations (CVs) were <10% and <12%, respectively. The serum concentration of myeloperoxidase was determined using ELISA kits (AESKULISA, Germany) with the intra- and inter-assay CVs of 3 and 3.7. The concentration of serum total antioxidant capacity was also assessed by ELISA kits (ZellBio GmbH, Ulm, Germany). The intra- and inter-assay CVs for TAC were 3.4% and 4.2%, respectively. The serum concentration of hs-CRP was determined by ELISA kits (Monobind Inc., USA). The CV for serum hs-CRP was 4%.

Dietary intakes

Dietary intake as a confounding factor could affect blood oxidative stress and inflammatory markers, so it was necessary to compare the two groups regarding these factors during the study. Therefore, dietary intakes of subjects were assessed using a 3-day dietary recall (for two consecutive days followed by a day of rest) at baseline and at the end of 12 weeks. The dietary intake of patients was analysed using Nutritionist IV software (Version 4.1, First Databank., The Hearst Corp., San Bruno, CA, USA) and the data from the United States Department of Agriculture Food Composition Table, which was modified for the Iranian foods (Ghafarpour *et al.* 1999).

Physical activity

At the beginning and the end of the study, physical activity was assessed by the use of the short form of the International Physical Activity Questionnaire (IPAQ), including seven questions related to physical activity associated with work, homework, and leisure time during the past seven days. Total metabolic equivalent of task (an hour per week) was calculated. The validity and reliability of the questionnaire had previously been confirmed in Iran (Fesharaki and Azad 2011).

Anthropometric indices and blood pressure

At baseline and after 12 weeks, participants *were* weighed in a fasted state *wearing* only *light clothing*, without *shoes*, and height *was measured* using a stadiometer with an accuracy of 0.1 cm in standard mode (without shoes and the shoulders, hips and heels were in contact with the wall). Finally, the body mass index (BMI) was calculated by dividing weight in kilograms by height in metres squared. Following a five-minute rest, diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured while the participants were in the sitting position with legs uncrossed and the forearm at the same level as the heart.

Statistical analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) for Windows version 20. The chi-square test was used to compare qualitative variables between the two groups. The Kolmogorov–Smirnov test was used to assess the normal distribution of quantitative data. We used *t*-test and paired *t*-test for normally distributed data to compare parameters between and within groups, respectively. The analysis of covariance (ANCOVA) was employed to control potential confounding variables. To compare changes between and within groups for non-normal distribution data set, Mann–Whitney *U* and Wilcoxon test were used. In addition, as dietary and anthropometric parameters were measured three times during the study, the analysis of variance for repeated measurements was used to compare data at various times. The results were expressed as mean ± SD, and differences were considered significant at $p < .05$. The analyses were conducted using an intention-to-treat approach (Wright and Sim 2003).

Results

Seventy-five individuals were enrolled in the study, of whom 12 were excluded due to non-compliance and/or withdrawal of consent (Figure 2). Ultimately, 63 participants, 13 (20.6%) females and 50 (79.4%) males, with an average age of 59.41 ± 8.44 years completed the study. Adherence to the study was 84% for all 63 participants. Based on the intention-to-treat (ITT) analyses included all participants ($n = 76$). One patient in the placebo and 2 patients in the intervention group reported symptoms including gastrointestinal problems. The results of the study indicate that LC was well tolerated by the majority of participants.

The two groups did not differ significantly with regard to age, gender and cigarette smoking ($p > .05$; Table 1). The mean age of participants was 59.7 (9.1) and 59.1 (7.1) years in the LC and placebo group, respectively, with no statistically significant difference between the groups ($p = .724$). There were no significant differences between the two groups in terms of physical activity at baseline ($p = .107$) and at the end of study ($p = .104$) and most of the participants in both groups had moderate levels of physical activity.

Dietary intake data (macronutrient distribution {as percentage of calories} changes and dietary fiber) and some micronutrients of the patients are summarised in Table 2. There were no significant differences in the mean dietary intake of total energy, protein, carbohydrate, fibre, total fat, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), cholesterol and total of vitamins E, C, selenium and β-carotene between the two groups at baseline and after 12 weeks ($p > .05$). In addition, these factors did not significantly change within each group during the study ($p > .05$; Table 2).

Patients in the two groups did not have a significant difference in the type and dosages of the used medications and were matched (Table 3).

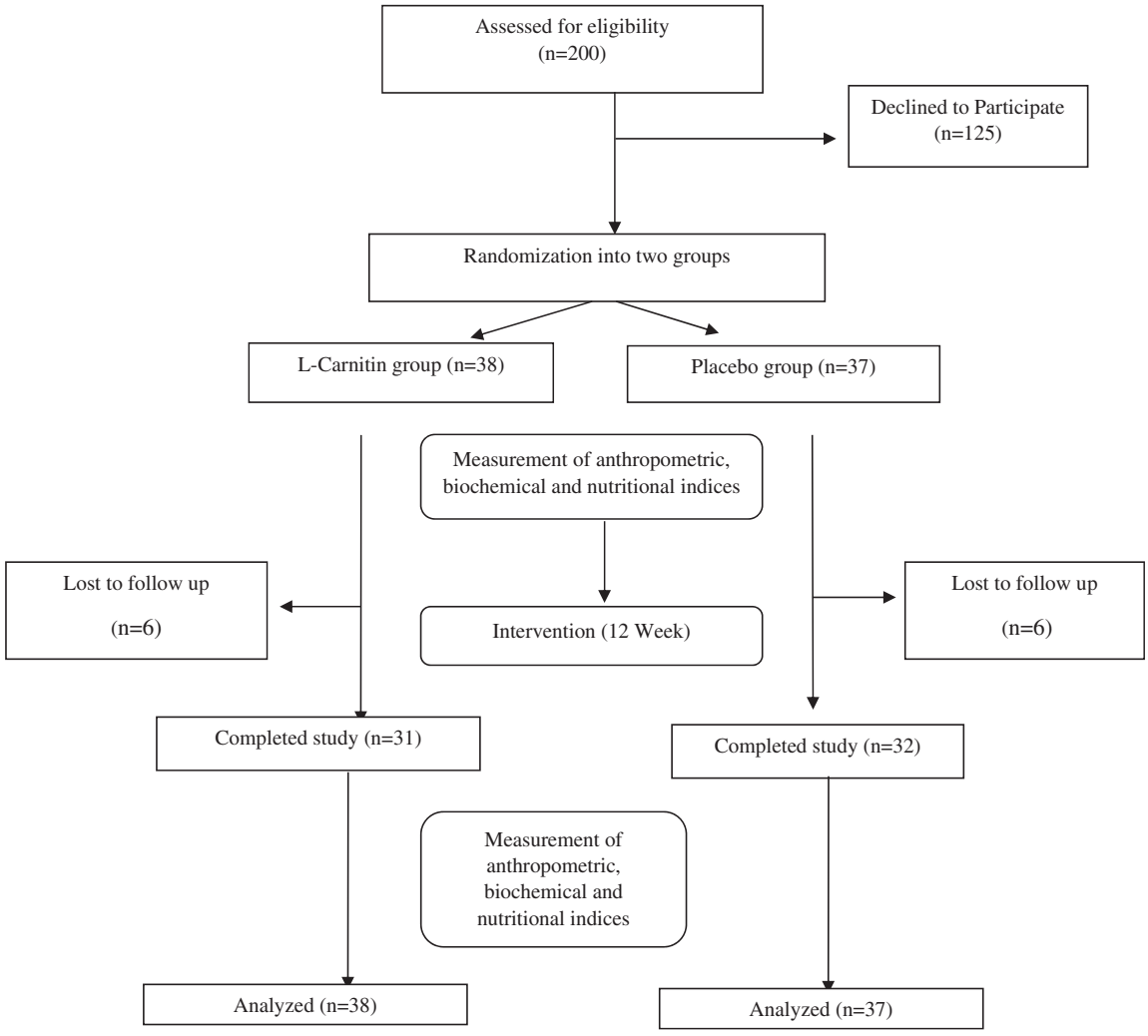

Figure 2. Flowchart of study.

Table 1. General characteristics of the study subjects.

Variable	L-Carnitine ($n=38$)	Placebo group ($n=37$)	p Value
Age (years)[a]	59.7 (9.1)	59.1 (7.8)	.724[b]
Sex[c]			.399
Male	29 (76)	25 (67)	
Family history of CAD	12 (31.9)	14 (37)	.935
Smoking	10 (26)	7 (18)	.134[d]
PAL			.107[d]
Low	8 (25)	10 (32)	
Moderate	14 (43)	18 (59)	
High	10 (31)	3 (9)	
Education 0.860[d]			
Illiterate or lower than diploma	12	13	
Diploma	8	6	
Academic	2	3	
Adverse effects[c]	2	1	.488
Compliance rate (%)	90	95	.721

BMI: body mass index; CAD: coronary artery diseases; PAL: physical activity levels.
[a]Values are expressed as mean (SD).
[b]Independent samples t-test.
[c]Values are expressed as frequency (%).
[d]Chi-square test.

As presented in Table 4, weight, BMI, LBM, BFM, DBP and SBP decreased significantly by the end of the study in both LC and placebo groups. There was a statistically significant difference between the two groups after intervention in terms of BFM, and SBP ($p > .05$) and controlling confounding variables, including energy and baseline values. Moreover, inter-group analysis indicated that anthropometric indices decreased significantly in the intervention group.

Before the intervention, the serum hs-CRP, NT and MPO concentrations showed no significant differences between the groups ($p = .220$, $p = .119$, $p = .340$, respectively). After 12 weeks of LC supplementation, a significant increase in serum TAC (0.30 ± 0.18 vs. -0.14 ± 10 mmol/L, $p = .009$) and significant decrease in MPO concentrations (-0.16 ± 0.17 vs. $0.14 + 0.12$ U/ml, $p = .002$), NT levels (-100.03 ± 23.88 vs. $85.43 + 21.8$ nmol/L, $p = .012$) and hs-CRP (-0.67 ± 0.17 vs. $0.30 + 0.20$ mg/dl, $p = .765$) were detected following the supplementation with LC, compared to the placebo (Figure 3). Thus, taking LC resulted in a significant decrease in inflammatory and oxidative stress markers in comparison with the placebo (Table 5).

Discussion

The results of the study indicate that LC supplementation attenuates inflammatory and oxidative stress. These results

Table 2. Dietary intakes in the L-carnitine and the Placebo groups at baseline and after 12 weeks of intervention.

	L-Carnitine group ($n = 38$)			Placebo group ($n = 37$)				
Factors/groups	Baseline	After 12 week	P_1[a]	Baseline	After 12 week	P_1	P_2[b]	P_3[c]
Energy (kcal/day)	1673.87±222.94	1598.9±237.92	.478	1640.74±228.17	1610.9±187.74	.405	.562	.712
Protein (g/day)	61.28±12.21	65±13.96	.278	63.58±12.01	65.9±9.17	.376	.454	.554
Carbohydrate (g/day)	221.91±28.98	212.93±31.25	.119	219.38±32.58	213.83±29.23	.093	.747	.906
Total fat (g/day)	60.21±10.79	54.03±10.04	.101	57.35±10.12	54.12±9.59	.145	.282	.610
SAFAs (g/day)	14.87±1.93	15.53±1.29	.122	15.51±2.17	16.19±1.75	.055	.221	.093
MUFAs (g/day)	16.31±1.95	16.34±1.66	.908	16.38±1.68	18.2±7.43	.456	.872	.172
PUFAs (g/day)	27.77±2.07	27.64±1.99	.777	27.08±2.82	26.12±4.87	.078	.269	.108
Cholesterol (mg/day)	408.03±47.7	384.65±61.49	.097	399.77±35.18	375.15±51.11	.324	.435	.508
Vitamin C (mg/day)	29.12±4.1	27.75±3.18	.453	29.61±3.89	28.22±3.56	.887	.632	.583
Selenium (mg/day)	0.04±0.03	0.04±0.03	.091	0.05±0.03	0.03±0.03	.446	.208	.191
β-carotene (Ug/day)	5.83±1.73	6.31±2.01	.134	5.86±1.42	6.2±1.46	.071	.934	.801
Vitamin E (mg/day)	1.44±0.84	1.44±0.76	.067	1.51±0.78	1.89±1.16	.113	.777	.076
Fibre (g/day)	6.72±2.36	7.26±2.11	.119	5.75±1.76	6.51±2.13	.101	.073	.165

SAFA: saturated fatty acids; MUFA: mono unsaturated fatty acids; PUFA: poly unsaturated fatty acids.
All values are presented as mean ± SD.
[a]P_1 : p values denote significance of within-group changes (paired t-test).
[b]P_2 : p values denote significance of between-group difference in the baseline (t-test).
[c]P_3 : Adjusted for baseline values using the analysis of covariance (ANCOVA) test.

Table 3. Used Medications in the L-carnitine and the Placebo Groups[a].

Medications/Groups	L-Carnitine group	Placebo group	p Values[b]
Atorvastatin 20	21 (65.6%)	26 (83.9)	.096
Losartan 25	11 (34.4%)	14 (45.2%)	.382
Aspirin 80	26 (81.3%)	26 (83.9%)	.784
Plavix 75	24 (75%)	22 (71%)	.718
Metoprolol 50	15 (46.9%)	20 (64.5%)	.159
Propranolol 20	2 (6.3%)	3 (9.7%)	.672
Clopidogrel	1 (3.1%)	1(3.2%)	.999
Hydrochlorothiazide	1 (3.1%)	3 (9.7%)	.355
Nitroglycerin	9 (28.1%)	9 (29%)	.936
Captopril	1 (3.1%)	5 (16.1%)	.104
Sustac	3 (9.4%)	2(6.5%)	.999
Amlodipine	3 (9.4%)	4 (12.9%)	.708

[a]n (%).
[b]p values denote significance of between group different (Chi-squared test).

also showed that decrease in MPO, NT, hs-CRP levels and increase in TAC levels among CAD subjects are appropriate evidence to support the claim. LC supplementation had no significant effect on BMI. After 3 months of LC supplementation (1 g/day), the level of TAC increased by 61% and MDA activities decreased by 16%, MPO by 8.5%, NT by 75% and hs-CRP by 47%. There are many studies with controversial results on the effects of LC supplementation on anthropometric indices. In the current study, greater reduction was observed in anthropometric indices (LBM, BFM) in the probiotic group compared with the placebo group. The findings are in line with some previous studies. A systematic review and meta-analysis of randomised controlled trials (RCTs) on the effect of LC supplementation on body weight and composition revealed that LC had a modest effect on body weight, BMI and fat mass, especially in adults with overweight/obesity (Talenezhad *et al.* 2020). Briefly, there are many studies on the beneficial effects of LC on body weight regulation; these effects are usually attributed to the effect of LC on the oxidation of fatty acids in skeletal and cardiac muscle by transporting long-chain fatty acids into the mitochondria (Fathizadeh *et al.* 2019). In addition, LC plays a vital role in the regulation of body weight through influencing energy production, glucose metabolism and trapping acetyl groups (Wang *et al.* 2018, Dehghan Ba *et al.* 2019).

The data about the effect of LC on blood pressure has shown inconsistent results (Parvanova *et al.* 2018, Askarpour *et al.* 2019). In our study, we made a significant change in SBP in the LC group. In a study by Askarpour *et al.* (2019) significant changes of SBP were seen with consumption of LC supplements in systematic review and meta-analysis of randomised controlled trials. On the contrary, in a trial conducted on diabetic patients, 1.5 g LC for 6 months did significantly affect blood pressure (Parvanova *et al.* 2018). Numerous confounding factors including the type of subjects, level of blood pressure, and dietary changes during intervention have been identified that might explain the varied results arising from different studies (Askarpour *et al.* 2019). The main result of the current study is that patients with CAD receiving LC supplements over 3 months show a significant decrease in some biomarker of inflammatory as compared with the placebo. In this study, after 12 weeks of LC supplementation (1000 mg/d), the serum concentration of MPO and NT, which are important markers of inflammation, showed a significant decrease from 1.11 U/ml to 0.94 U/ml (by 15%) and from 369.51 nmol/L to 257.46 nmol/L (by 23%), respectively. Consistent with the present trial, Lee *et al.* (2014) found a significant decrease in inflammatory markers among patients with CAD following the intake of 1 g LC for 3 months. Additionally, numerous reports advocated that LC supplementation had significant effect on inflammatory markers in many clinical settings (Lee *et al.* 2015, Lee *et al.* 2016, Singhai *et al.* 2017). In contrast, Volek *et al.* (2008) stated that 2 g/day LC (L-Carnitine L-Tartrate) for 3 weeks had no significant effect on plasma tumour necrosis factor-alpha in healthy young adults. The uncertainty in the results of studies might be caused by various research designs and different doses of LC along with intervention period. CAD is a multifactorial disease, and inflammatory process is defined as a major risk factor for CAD (He and Zuo 2015). In patients with CAD, reducing serum MPO and NT concentrations may have an important role in preventing subsequent side effects. Studies have shown that increasing the concentration of MPO can cause plague vulnerability (Teng *et al.* 2017). Serum levels of MPO were significantly elevated in

Table 4. Anthropometric indices and blood pressure in L-carnitine and placebo group at baseline and after 12 weeks of intervention.

	L-Carnitine group ($n = 38$)			Placebo group ($n = 37$)				
Variable	Baseline	After 12 weeks	P_1[a]	Baseline	After 12 weeks	P_1[a]	P_2[b]	P_3[c]
Weight (kg)	74.16±8.94	73.50±9.67	.217	74.72±9.51	74.46±10	.41	.793	.575
BMI (kg/m^2)	26.4±2.33	26.15±2.5	.178	27.65±2.4	27.55±2.68	.398	.026	.068
LBM (kg)	54.04±8.3	57.68±10.35	<.001	54.64±8.74	54.79±8.84	.77	.762	.112
BFM (kg)	19.25±4.36	15.39±4.66	<.001	24.33±5.74	31.52±32.71	.197	<.001	.035
SBP (mmHg)	121.18±16.27	112.24±11.81	<.001	132.38±24.05	129.32±18.11	.189	.021	<.001
DBP (mmHg)	76.55±9.48	75.73±8.1	.533	82.64±12.88	79.13±13.19	.043	.76	.172

$p < .05$ is considered significant.
[a]P_1 : p values denote the significance of within-group changes (paired t-test).
[b]P_2 : p values denote significance of between-group difference in the baseline (t-test).
[c]P_3 : Adjusted for baseline values and energy intake changes using the analysis of covariance (ANCOVA) test.

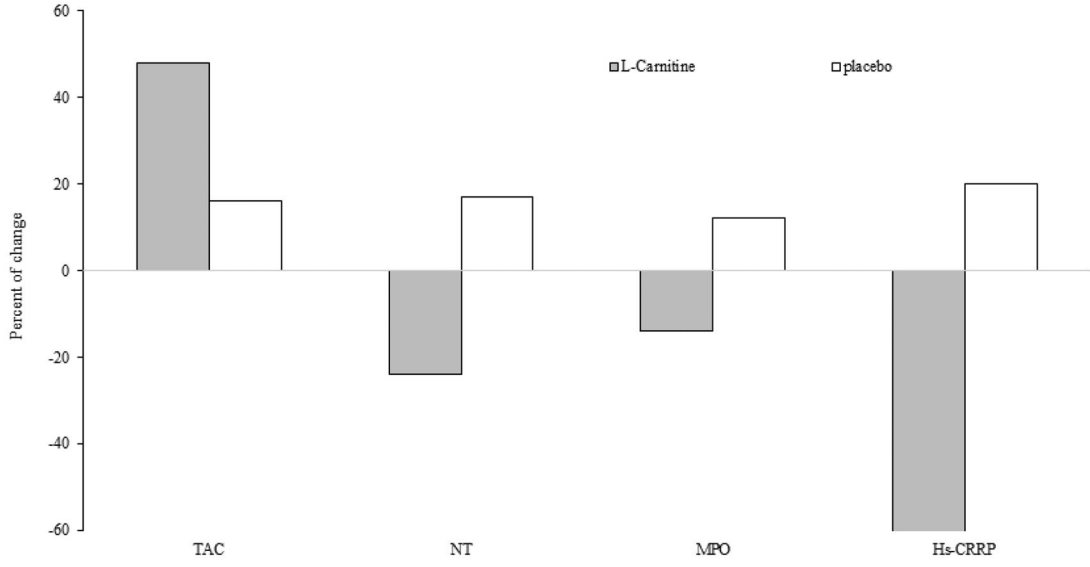

Figure 3. Percentage change of inflammatory markers.

Table 5. Effect of L-carnitine on inflammatory and oxidative stress biomarkers.

	L-Carnitine group ($n = 38$)			Placebo group ($n = 37$)				
Variable/Groups	Baseline	After 12 weeks	P_1[a]	Baseline	After 12 weeks	P_1[a]	P_2[b]	P_3[c]
TAC (Mm/L)	0.66±0.21	0.98±0.23	.001	0.83±0.42	0.69±0.42	.131	.096	.009
NT (nmol/L)	413.89±222.86	313.86±247.5	.027	478.03±474.22	563.28±477.14	.119	.45	.012
MPO (U/ml)	1.1±0.5	0.94±0.47	.015	1.16±0.41	1.3±0.48	.340	.62	.002
hs-CRP (mg/dL)	1.18±0.33	0.51±0.5	.002	1.45±0.38	1.75±0.56	.220	.36	.001

$p < .05$ is considered significant.
[a]P_1 : p values denote the significance of within-group changes (paired t-test).
bP_2 : p values denote significance of between-group difference in the baseline (t-test).
cP_3 : Adjusted for baseline values, weight, and energy intake changes using the analysis of covariance (ANCOVA) test.

individuals who experienced sudden cardiac death (Osman *et al.* 2019). MPO by nitrosylation the tyrosine residues of lipoprotein A-I component in high-density lipoprotein can interfere with cholesterol efflux from macrophages (Chistiakov *et al.* 2017). Accordingly, plasma nitrosylated HDL levels in patients with CAD are approximately twofold higher than those in healthy subjects (Bhutani and Tangadi 2020). MPO also produces active nitrogen species that can convert low-density lipoprotein (LDL) into an atherogenic form. The modified LDL is endocytosed by macrophages and leads to the formation of foam cells (Doodnauth *et al.* 2019). The accumulation of MPO and nitrotyrosine in the subendothelial space of arterioles has been linked to the greater role of MPO and NT in inflammatory diseases such as CAD. The measurement of free NT is a proper approach to assessment of nitroxidative stress (Pourfarzam *et al.* 2013).

In addition, MPO is a member of the superfamily of peroxidase enzymes, which plays a major role in inflammatory and oxidative stress processes (Ndrepepa 2019). Recently, this enzyme has been considered as a potential biomarker for CVDs, as well as a potential target for treatment. Findings suggest that MPO be involved in extensive oxidative changes in the tunica intima of the coronary artery and indicate that MPO has a definite role in the pathophysiology of coronary

artery disease (Ou *et al.* 2017). It is now clear that NT as a product of MPO has important implications in CAD (Ramachandra *et al.* 2020). Immunohistochemistry studies have revealed the increase of NT in the athermanous plaque and confirmed the role of NT in atherosclerosis (Ebrahimian *et al.* 2018, Bhutani and Tangadi 2020). As far as we know, no clinical trial had appraised the effects of LC attenuate inflammatory markers including MPO and NT in patients with CAD, so this study is the first work carried out in this field to date. The effects of LC supplementation on some markers of oxidative stress and systemic inflammation such as MDA, hs-CRP, IL-6 and TNFα in CAD patients showed that LC could reduce these markers (Singhai *et al.* 2017).

In this study, the concentration of serum hs-CRP significantly reduced from 1.18 mg/dl to 0.51 mg/dl in the LC group. In contrast, in the placebo group, its concentration increased significantly, and the difference in the means of two groups was significant. As a result, it can be claimed that the LC supplementation has anti-inflammatory properties in CAD patients. In cardiovascular patients, inflammation is a common complication, and inflammatory factors have been found to be higher in CAD patients than the healthy subjects (Granger and Kvietys 2015). CRP plays a pivotal role in many aspects of atherogenesis, including activation of complementary pathways, macrophage lipid uptake, the release of pro-inflammatory cytokines, induction of tissue texture expression in monocytes, increase of endothelial dysfunction and inhibition of nitric oxide production (Shrivastava *et al.* 2015). In a study conducted on patients with CAD, supplementation with LC at a dose of 1000 mg/day for 12 weeks significantly decreased systemic inflammatory factors, including CRP and TNF (Lee *et al.* 2015). Furthermore, many clinical trials have shown that LC can reduce CRP levels in different clinical settings (Dehghan Ba *et al.* 2019)). However, it has been reported that LC supplementation (2000 mg/d) has no significant effect on the markers of inflammation in healthy individuals (Volek *et al.* 2008). This result can be attributed to this fact that serum levels of inflammatory factors are not high in healthy people. In our study, 35% of participants had high inflammatory status (based on serum hs-CRP level ($\geq$1 mg/L), which significantly decreased by 47% after 12 weeks of intervention. The findings also indicate that the cardiovascular health benefit of LC supplementation was the result of decreased hs-CRP levels and other inflammatory markers.

The evidence about the effect of LC supplementation on oxidative stress has shown inconsistent results. Similar to some previous studies, in the current trial, TAC levels were observed to be higher in the LC group compared with the placebo group. In a study done on CAD patients, L-carnitine (1000 mg/day for 12 weeks) increased the activities of catalase by 16%, superoxide dismutase by 47% and glutathione peroxidase by 12% (Ramachandra *et al.* 2020). Lee *et al.* (2014) reported that 1 g/day of LC for 3 months was associated with a significant increase in TAC levels in CAD patients. On the contrary, in a trial conducted on healthy young adults, 2000 mg LC for 5 weeks failed to affect oxidative stress (Volek *et al.* 2008). In the present study, we did not observe statistically significant changes in nutrient intakes and physical activity during the intervention, so these effects are usually attributed to LC supplementation. The main mechanisms by which LC administration might lower oxidative stress are not well understood. LC has the potential to inhibit oxidative stress and regulate oxidative nitric stress (Volek *et al.* 2008). L-Carnitine can protect mitochondria function by affecting the catalase and superoxide as antioxidant enzymes (Le Borgne *et al.* 2017). Some evidence has shown that the level of LC in ischaemic heart disease decreases in cardiac muscle (Wang *et al.* 2018). Any reduction in antioxidant enzymes can accelerate the progression of atherosclerosis (Ou *et al.* 2017). Increasing the activity of the antioxidant enzymes of the red blood cells can protect endothelial cells from oxidative damages (Alvarez de Sotomayor *et al.* 2007). L-Carnitine is known to be an effective antioxidant agent in cardiovascular diseases (Scioli *et al.* 2019). LC may quench the activity of some free radicals such as superoxide and hydrogen peroxide and may interfere with the formation of other free oxygen species and chelation of ferrous ions (Scioli *et al.* 2019). In the LC molecule, the carbonyl group can stabilise free radicals formed on α-carbon, and protect the plasma components against the toxic activity of active oxygen species and active nitrogen species (Ribas *et al.* 2014, Scioli *et al.* 2019).

Some limitations of the present study should be noted. First, the sample size of this study was small. Second, this study was designed using daily LC supplements for three months only. Therefore, larger and longer randomised clinical trials are recommended to confirm these findings. Further studies are needed to measure other indicators of oxidative stress, inflammatory markers and factors affecting endothelial function, including oxidised low-density lipoprotein (ox-LDL), which affects cardiovascular function.

In conclusion, the findings of this study indicated the effectiveness of L-carnitine as an auxiliary treatment for increasing antioxidant activity and reduction of oxidative stress indices and systemic inflammatory factors in CAD patients. Patients with CAD may benefit from these beneficial effects by using LC supplements.

Ethical approval

The trial was given ethical approval by the Ethics Committee of the Deputy of Research and Technology of Kermanshah University of Medical Sciences (ethics approval number: KUMS.REC.1395.80) and registered with the Iranian Clinical Trials Registry (registration number IRCT2016060528260N1).

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Disclosure statement

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