Nutrition 31 (2015) 475–479

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Antiinflammatory effects of L-carnitine supplementation (1000 mg/d) in coronary artery disease patients

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ARTICLE INFO

Article history:
Received 6 June 2014
Accepted 5 October 2014

Keywords:
L-carnitine
Inflammation
Antioxidant
Supplement
Coronary artery disease

ABSTRACT

Objective: Inflammation mediators have been recognized as risk factors for the pathogenesis of coronary artery disease (CAD). The purpose of this study was to investigate the effect of L-carnitine supplementation (LC, 1000 mg/d) on inflammation markers in patients with CAD.
Methods: We enrolled 47 patients with CAD in the study. The patients with CAD were identified by cardiac catheterization as having <50% stenosis of one major coronary artery. The patients were randomly assigned to the placebo (n = 24) and LC (n = 23) groups and the intervention was administered for 12 wk. The levels of LC, antioxidant status (malondialdehyde and antioxidant enzymes activities), and inflammation markers (C-reactive protein [CRP], interleukin [IL]-6, and tumor necrosis factor [TNF]-α) were measured.
Results: Thirty-nine participants completed the study (19 placebo; 20 LC). After LC supplementation, the levels of inflammation markers were significantly reduced compared with the baseline (CRP, $P < 0.01$; IL-6, $P = 0.03$; TNF-α, $P = 0.07$) and those in the placebo group (CRP, $P < 0.05$; IL-6, $P = 0.04$; TNF-α, $P = 0.03$). The levels of inflammation markers were significantly negatively correlated with the levels of LC and antioxidant enzymes activities ($P < 0.05$).
Conclusions: We suggest that LC supplementation, due to its antioxidant effects, may have potential utility to reduce inflammation in CAD.

Introduction

Inflammation and atherosclerosis have become well established over the past decade. Many theories describing the atherosclerotic disease process are correlated with inflammation status [1,2]. Mounting evidence indicates that a higher inflammation status plays a key role in development of coronary artery disease (CAD) [1,2]. In clinical studies, the levels of C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α were commonly used as inflammation markers to predict the risk for CAD [3–5]. In the inflammatory cascade of atherosclerosis, the proinflammatory risk factors such as oxidized low-density lipoproteins and proinflammatory cytokines (e.g., TNF-α) may induce IL-6 secretion. IL-6 is a messenger cytokine that is secreted by macrophages and smooth muscle cells in the atherosclerotic lesion. Then, CRP is released as a product of hepatic stimulation and inflammation, under the regulation of IL-6. Finally, systemic inflammation is triggered and results in elevated levels of inflammation markers attributed to atherosclerosis [4].

L-carnitine (β-hydroxy-γ-trimethyl-amino-butyric acid; LC) is an essential compound that is synthesized from lysine and methionine [6]. LC assists in the β-oxidation of long-chain fatty acids and transports fatty acids into the mitochondrial matrix [6,7]. Recently, an in vitro study demonstrated that LC plays a critical role in inflammatory diseases by modulating inflammatory cell functions [8]. LC might have the potential to control inflammation by reduction of major inflammatory cytokines,

This study was supported by a grant from the Chung Shan Medical University (CSMU-INT-101-09), Taiwan. BJL carried out the study, performed the data analyses, and drafted the manuscript. JSL and YCL carried out the study and sample analyses. PTL conceived the study, participated in its design, and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

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http://dx.doi.org/10.1016/j.nut.2014.10.001
0899-9007/

including nuclear factor-kappa B (NF-κB) and TNF-α [8,9]. Most clinical trials have examined antiinflammatory effect of LC supplementation at doses of 1000 to 1200 mg/d in the patients on hemodialysis [10–13], but not all of them have shown an effect on inflammation markers [13]. To our knowledge, there is no published information about the antiinflammatory effect of LC supplementation in patients with CAD. The Ministry of Health and Welfare in Taiwan recommends a daily dietary intake of ≤2000 mg of LC. It is worthwhile to investigate whether LC supplement shows an antiinflammatory effect in patients with CAD. Thus, we tested a dose of 1000 mg/d in patients with CAD and examined whether this dose could be a dietary supplement for daily use. The purpose of this study was to investigate the effect of LC supplements (1000 mg/d) on inflammation markers in patients with CAD.

Materials and methods

Participants

This study was designed as a single-blinded, randomized, parallel, placebo-controlled study. Patients with CAD were recruited from the cardiology clinic of Taichung Veterans General Hospital, which is a teaching hospital in central Taiwan. CAD was identified by cardiac catheterization as having <50% stenosis of one major coronary artery or receiving percutaneous transluminal coronary angioplasty. Patients with diabetes, liver, or renal diseases were excluded to minimize the influence of other cardiovascular risk factors. Patients undergoing statin therapy or currently using vitamin supplements were also excluded. Informed consent was obtained from each participant. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital, Taiwan and registered at ClinicalTrials.gov (NCT01819701).

With a sample size calculation, we expected that the change in the levels of antioxidant enzymes activities would be 5.0 ± 7.0 U/mg of protein after LC supplementation; therefore, the desired power was set at 0.8 to detect a true fact and at an α value = 0.05 with a minimum sample size of 18 in each intervention group. We enrolled 47 patients with CAD in this study and randomly assigned them to the placebo (n = 24) or LC (n = 23) group. The LC and placebo (starch) capsules were commercially available preparations (New Health Taiwan Co., Ltd.). The intervention was administered for 12 wk. The participants were instructed to take two capsules (500 mg) daily, equal to 1000 mg/d. To monitor compliance, the researchers reminded participants to check the capsule bag every 4 wk to confirm that it was empty, and we measured the serum LC level before and after intervention. Participants' age, blood pressure, smoking, drinking, and exercise habits were recorded. Body weight, height, and waist circumferences were measured and the body mass index (kg/m^2) was calculated.

Blood collection and biochemical measurements

Fasting venous blood samples (15 mL) were obtained to estimate the hematologic and vitamin status. Blood specimens were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) that contained EDTA as an anticoagulant or that contained no anticoagulant as required. Serum and plasma were prepared after centrifugation (3000*g*, 4°C, 15 min) and were then stored at −80°C until analysis. Hematologic parameters (serum creatinine, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol) were measured by an automated biochemical analyzer (Hitachi-7180 E, Tokyo, Japan). Plasma malondialdehyde (MDA) was determined using the thiobarbituric acid reactive substances method, as previously described [14]. Serum was diluted with 400 × sample diluent for LC measurement. Serum level of LC was measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Cusabio, Wuhan, China) according to the supplier's instructions and the detection range for the analysis of LC were 0.8 to 50 μmol/L. The intra- and inter-assay precision were <8% and <10%, respectively.

Serum level of CRP was quantified by particle-enhanced immunonephelometry with an image analyzer (Dade Behring, Deerfield, IL, USA). Serum levels of IL-6 (eBioscience, San Diego, CA, USA) and TNF-α (R&D Systems Inc., McKinley Place NE, Minneapolis, USA) were measured by ELISA using a commercially available kit. The detection range for IL-6 was 1.1 to 14.3 pg/mL and for TNF-α was 0.5–16 pg/mL. The intra- and inter-assay precisions of IL-6 were 4.9% and 6%, respectively. The intra- and inter-assay precisions of TNF-α were 4.3% and 7.2%, respectively.

Red blood cell (RBC) samples were washed with normal saline after removing the plasma. Then, the RBCs were diluted with 25 × sodium phosphate buffer for superoxide dismutase (SOD) and glutathione peroxidase (GPx) measurements, and with 250 × sodium phosphate buffer for catalase (CAT) measurement. The antioxidant enzymes activities (CAT, SOD, and GPx) were determined using fresh samples and the methods for measuring these activities have been described previously [15–17]. The protein content of the plasma and RBCs was determined based on the biuret reaction of the bicinchoninic acid kit (Thermo, Rockford, IL, USA). The values for the antioxidant enzymes activities were expressed as units/mg of protein. All of the analyses were performed in duplicate.

Statistical analyses

The data were analyzed using the SigmaPlot software package (version 12.0, Systat, San Jose, CA, USA). The normal distribution of variables was tested by the Kolmogorov-Smirnov test. Differences in participants' demographic data and the hematologic measurements between the placebo and LC groups were analyzed by Student's *t* test or the Mann-Whitney rank-sum test. The paired *t* test or Wilcoxon signed rank test was used to analyze the data within each group before (baseline) and after intervention (week 12). For the categorical response variables, differences between the two groups were assessed by the χ^2 test or Fisher's exact test. To examine the correlations between the levels of inflammation markers, oxidative stress, and antioxidant enzymes activities after LC supplementation, the Pearson product moment correlations were used. The results were considered statistically significant at $P < 0.05$. Values presented in the text are means ± SD.

Results

Study participant characteristics

The sampling and trial profiles are summarized in Figure 1, along with the number of participants who completed the study in each group. Table 1 presents the demographic data and the health characteristics of the participants at the baseline. There were no significant differences between the two groups with respect to age, blood pressure, anthropometric measurements, hematologic entities (serum creatinine and lipid profiles), and the frequency of smoking, drinking, or exercise at baseline.

Effects of LC supplementation on levels of inflammation markers

The levels of inflammation markers after supplementation are shown in Table 2. After 12 wk of LC supplementation, the participants in the LC group had significantly lower levels of inflammation markers than at baseline (CRP, $P < 0.01$; IL-6, $P = 0.03$, TNF-α, $P = 0.07$) and than those in the placebo group (CRP, $P < 0.05$; IL-6, $P = 0.04$, TNF-α, $P = 0.03$). The changed levels of CRP ($P = 0.04$), IL-6 ($P < 0.05$), and TNF-α ($P = 0.04$) were significantly lower in the LC group than in the placebo group.

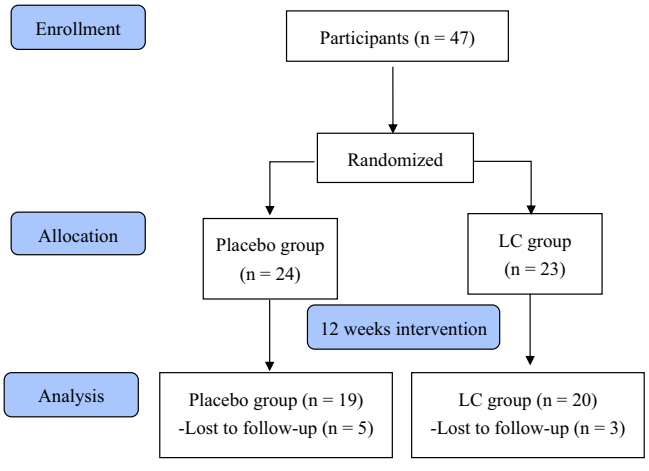

Fig. 1. Flow diagram. LC, L-carnitine.