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ORIGINAL ARTICLE

Effects of L-carnitine supplementation on biomarkers of oxidative stress, antioxidant capacity and lipid profile, in patients with pemphigus vulgaris: a randomized, double-blind, placebo-controlled trial

H Mohammadi[1], M Djalali[1], M Daneshpazhooh[2], NM Honarvar[1], C Chams-Davatchi[2], F Sepandar[1], Z Fakhri[1], E Yaghubi[1], M Zarei[1] and MH Javanbakht[1]

BACKGROUND/OBJECTIVES: Pemphigus vulgaris (PV), as an autoimmune disease including mucosa and the skin, is associated with several complications and comorbidities. The present study planned to determine the effect of L-carnitine (LC) supplementation on biomarkers of oxidative stress (OS), antioxidant capacity and lipid profile in PV patients.

SUBJECTS/METHODS Fifty two control and patients with PV, participated in the current randomized, double-blind, placebo-controlled clinical trial. The patients were allocated randomly to receive 2 g per day LC tartrate subdivided into two equal doses of 1 g before breakfast and dinner ($n = 26$) or placebo ($n = 26$) for 8 weeks. Anthropometric, lipid profile and OS values were determined at baseline and end of intervention period.

RESULTS: LC intake significantly reduced serum levels of triglycerides, total-, LDL- cholesterol and oxidative stress index (OSI; $P < 0.05$). In addition, supplementation with LC resulted to a meaningful increase in levels of total antioxidant capacity (TAC) ($P = 0.05$) and serum carnitine ($P < 0.001$). LC intake revealed non-significant change in serum total oxidant capacity ($P = 0.15$) and HDL- cholesterol ($P = 0.06$) in comparison to the placebo.

CONCLUSIONS: LC consumption may have favorable results on TAC, OSI and lipid profiles in patients with PV. The results were in line with the idea that LC supplementation can be associated with positive effects on metabolic status and OS of patients with PV.

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INTRODUCTION

Pemphigus vulgaris (PV) is a scarce autoimmune blistering disorder of the skin and/or mucous membranes. Corticosteroids and immunosuppressive drugs are the most commonly used drugs in treatment of PV.[1] Long-term use of such treatment may lead to hypertension, osteoporosis, diabetes mellitus and susceptibility to infections.[2] Also, based on the several studies, there is a positive association between corticosteroid consumption and obesity, insulin resistance,[3] hypertension and hyperlipidemia.[4] Therefore, finding new ways to reduce the PV complications is needed.

L-carnitine (LC), a small water-soluble compound, is synthesized endogenously from lysine and methionine or acquired from animal foods such as milk and meat.[5] LC as a substantial cofactor for beta-oxidation of fatty acids plays a serious role in the transportation of fatty acids into the matrix of the mitochondrion.[6] Prior researches revealed that treatment with LC had beneficial effects on metabolic syndrome,[7] insulin resistance,[8] hypertension,[9] and hyperlipidemia.[10] Recently, in a study by Malek Mahdavi *et al.*,[11] it was found that taking 750 mg per day oral LC could significantly alleviate the pain and decreased the serum inflammatory mediators in women with knee osteoarthritis. Furthermore, another study[6] suggested that LC supplementation could be a suitable treatment strategy for those suffering obesity, hypertension and insulin resistance.

Oxidative stress (OS) takes places during the many physiological functions such as mitochondria and phagocytosis. Overproduction of reactive oxygen species (ROS) in the phagocytosis of phagocytic cells was neutralized by antioxidant defense system.[12–14] OS which is specified as imbalance between ROS generation and the antioxidant system is associated with several diseases.[14,15] Previous reports suggested that PV patients have higher OS indicators[16] and lower antioxidant capacity.[15] With increasing body of evidence, a pivotal role of LC supplements was revealed in amelioration of parameters of OS and antioxidant defence.[17,18] Therefore, the LC supplementation may have several benefits on metabolic status of subjects with PV and it is necessary to detect new strategies aimed at improving a metabolic status and OS parameters in PV patients. Thus, the purpose of the current study was to investigate the effect of LC consumption on biomarkers of OS, antioxidant capacity and lipid profile, in PV patients.

MATERIALS AND METHODS

Participants

All participants aged 30–65 years with PV diagnosis based on the clinical, histological and immunological criteria[19] were recruited during August

[1]Department of Cellular and Molecular Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran and [2]Autoimmune Bullous Diseases Research Center, Department of Dermatology, Tehran University of Medical Sciences, Tehran, Iran. Correspondence: Dr MH Javanbakht, Department of Cellular and Molecular Nutrition, School of Nutrition Sciences and Dietetics, Tehran University of Medical Sciences, Poorsina Street, Enghelab Avenue, Tehran 14155-6446, Iran.
E-mail: mhjavan2001@yahoo.com
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2015 to January 2016 from the Pemphigus clinics of Razi Hospital, Tehran University of Medical Sciences (TUMS), Tehran, Iran. Our primary outcomes were OS biomarkers and secondary outcomes were lipid profile and anthropometric indices. To compute the sample size, we applied parallel-design randomized controlled trial formula based on 0.05 for type one (α) error and 0.20 for type two error (*β*). With regard to past study,[16] we considered 96.18 as standard deviation (s.d.) and 86.2 as the effect size (*d*) of total oxidant capacity (TOC) as the principal outcome variable. So, we required 20 patients per group. However, 26 patients were recruited per group to take into account the plausible dropouts. The cases were enrolled in the study if they met these inclusion criteria: (1) At least one year with the disease; (2) Using corticosteroid alone or with methotrexate, azathioprine or mycophenolate mofetil drugs; (3) BMI < 35; (4) Not having a history of diabetes, cardiovascular disease, renal, hepatic and inflammatory disorders; (5) being a non-smoker, (6) Not taking antioxidant supplements within the past 3 months before the intervention; (7) Not taking lipid-lowering medications and drugs which have obvious interaction with carnitine or influence its metabolism, that is, theophylline or valproate. The study came after the Declaration of Helsinki and written informed consent was completed by the all subjects after confirmation the trial by the TUMS ethical committee. The study protocol of present trial was approved by Iranian registration of clinical trials (IRCT code: IRCT2015062322769N4).

Study design

This was a randomized, placebo-controlled, double-blind parallel-group clinical trial. Participants were matched for age and gender and then randomly allocated to receive 2 g LC tartrate per day ($n = 26$) or placebo ($n = 26$), subdivided into two equal doses of 1 g before breakfast and dinner for 8 weeks. Due to scarcity of evidenced-based appropriate dosage for LC in PV patients, we used the suggested doses according to the past study in diabetic patients.[10] Production of LC supplements and placebo capsules (microcrystalline cellulose) were done by Asal Daroo Kish Pharmaceutical Company in Kish, Iran. LC supplement and its placebo were in the same appearance such as color, shape, size and packaging, which coded by the company to assurance blinding. Stratified randomization was used to allocate subjects to a main drug and placebo. Researchers not informed about randomization process until completion of data analyses (concealment). As well, during the study, no researchers and patients were aware of the drugs randomly allocated. Randomized allocation and participants group assignment was done by study technician. The participants were asked not to change their medications. Compliance to the LC supplementation was affirmed via serum LC measurement. Researchers controlled all patients for any possible side effects through phone interviews weekly.

Clinical assessment

Harman scores were used for disease severity evaluation.[20] For curtailing observer bias Participants were assessed by a single expert physician. The severity of oral mucosa and skin involvement was measured by a simple scoring system of 0–3 (0, quiescent; 1, mild; 2, moderate; 3, severe). Body weight and height were determined by a digital scale (to the nearest 0.1 kg) and stadiometer (to the nearest 0.1 cm) respectively (Seca, Hamburg, Germany). Waist circumference (WC) was assessed with a non-stretching tape measure (Seca; Germany) at the midpoint between the costal margin and the iliac crest with no pressure on the body surface. BMI was computed as weight (kg) divided by height^2 (m^2). All patients' medical and drug history were obtained.

Dietary intake and physical activity assessments

For evaluating physical activity level before and after intervention we used an international physical activity questionnaire (IPAQ). Based on the grade scoring protocol of the short form of IPAQ we grouped our participants into high, moderate or low physical activity level.[21] For better comparison dietary intakes controlled by 24- hour recall method for 3 days (including 2 working days and 1 weekend day) at the beginning and the end of trial. The dietary recalls were analyzed using Nutritionist IV software (First Databank, San Bruno, CA, USA) adjusted for Iranian foods. The participants were asked not to change their dietary habits and physical activity during the study.

Biochemical assessment

Fasting blood samples (10 ml) were collected at the beginning and the end of trial then immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3000 r.p.m. for 10 min to separate serum and stored at −80 °C until analysis. Serum levels of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were determined by enzymatic methods using commercial kits (Pars Azmoon, Tehran, Iran) and auto-analyzer system (Selectra E, Vitalab, Netherland). All inter- and intra-assay CVs for lipid profiles measurements were lower than 5%. Very Low density lipoprotein cholesterol (VLDL-C) was computed through the following formula: VLDL-C $=$ TC $-$ (LDL-C+HDL-C). Serum LC was measured by ELISA kit (Zellbio Gmbh, Ulm, Germany) and ELISA plate reader (Elx800, Bio-Tek Instrument Inc., Winooski, VT, USA) at a wave length of 450 nm. Serum total antioxidant capacity (TAC) and TOC were measured using assay kits (Zellbio Gmbh). The inter- and intra-assay CVs for LC, TOC and TAC were $< 5\%$. Oxidative stress index (OSI)[16] was computed through following formula: OSI $=$ [(TOC, μmol/l)/ (TAC, μmol/l) $\times$ 100].

Statistical analyses

Statistical analyses were conducted using SPSS, version 17 (SPSS Inc, Chicago, IL, USA). The analyses were performed on the basis of an intention-to-treat (ITT) approach. Missing values were treated according to Last-Observation-Carried-Forward method. All parameters were reported as mean ± s.d. Normal distribution of all definite parameters was tested by the Kolmogorov–Smirnov test. Log transformation was performed for non-normally distributed variables. Categorical variables compared by the Pearson chi-square test. Within group comparisons were done by paired-sample *t*-test. The Student's *t*-test was applied to find out any differences in baseline characteristics and dietary intakes between the trial groups. ANCOVA test was applied to detect any differences between supplementation groups at the end of the trial, adjusting for baseline values.

RESULTS

Among the total of the 52 PV patients who entered to the study, 2 patient in the LC group (due to personal reasons) and 3 patient in the placebo group (due to change in dose of prednisone ($n = 2$) and personal reasons ($n = 1$)) were withdrawn from study (Figure 1). Although the analyses was performed according to ITT approach, all 52 participants were enrolled in the final analyses. No adverse effects were reported following the LC intake or placebo in PV patients.

As presented in Table 1, at baseline, the demographic characteristics, disease severity, disease duration and physical activity of participants were not significantly varied between supplementation groups. The mean intake of prednisone was 9.51 ± 4.19 mg/day in the LC group and 11.25 ± 5.32 mg per day in the placebo group ($P > 0.05$). No significant baseline differences between two groups were seen concerning in type of medications ($P > 0.05$).

Dietary intake of study participants were shown in Supplementary Table S1. No within- or between-groups differences were observed for dietary intake of total energy, carbohydrates, proteins, fats, selenium, zinc, cholesterol, vitamins C and E.

Compared with the placebo, LC intake led to significant reductions in TC, TG, LDL-C, VLDL-C, OSI and a significant rise in carnitine concentration ($P < 0.05$). Interestingly, a marginally significant rise in TAC ($P = 0.05$) and HDL-C ($P = 0.06$) concentration were seen after taking LC supplementation compared with placebo (Table 2). Regarding weight, BMI, WC and serum TOC concentration, there were no significant differences between placebo and intervention groups. In addition, within-group comparisons also showed that the levels of TG ($P = 0.001$), TC ($P = 0.001$), LDL-C ($P = 0.003$), VLDL-C ($P = 0.001$), TOC ($P = 0.01$), and OSI ($P = 0.003$), significantly decreased and the mean concentration of LC ($P < 0.001$) and HDL-C ($P < 0.001$) significantly increased post intervention in LC group.

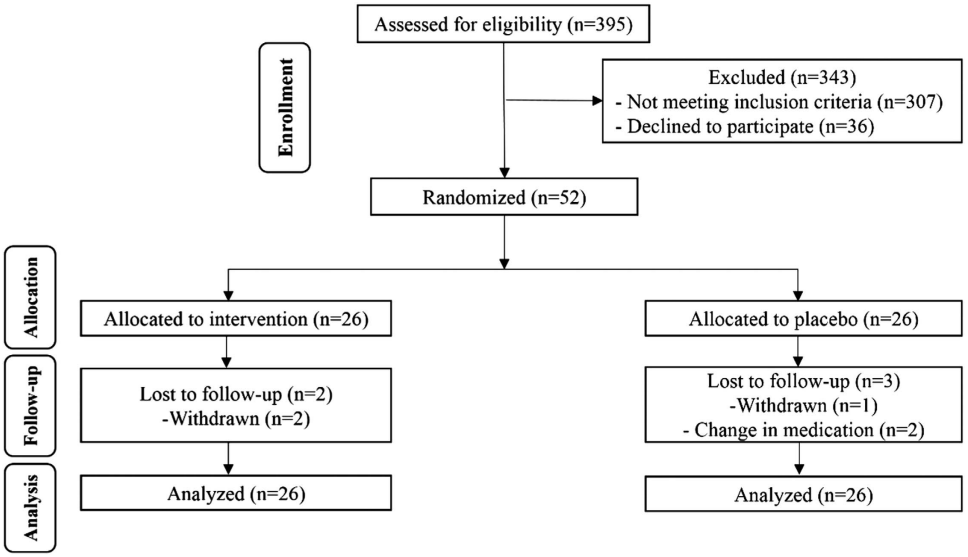

Figure 1. Patients' flow diagram.

Table 1. Baseline characteristics of study subjects[a]

Variable	*L-Carnitine group* (n = *26*)	*Placebo group* (n = *26*)	P-*value*[b]
Male/Female	10/16	10/16	1.00
Age (year)	41.04 ± 9.65	40.65 ± 9.90	0.88
Weight (kg)	77.70 ± 13.25	73.06 ± 9.32	0.15
Height (cm)	166.36 ± 8.44	165.88 ± 11.04	0.86
BMI (kg/m^2)	28.07 ± 4.33	26.66 ± 3.48	0.20
WC (cm)	94.85 ± 9.24	90.87 ± 8.81	0.12
Pemphigus duration (year)	1.71 ± 0.56	1.81 ± 0.60	0.54
Prednisone (mg per day)	9.51 ± 4.19	11.25 ± 5.32	0.19
Disease severity oral n *(%)*			0.27
Quiescent	9 (34.6)	10 (38.5)	
Mild	13 (50)	8 (30.8)	
Moderate	4 (15.4)	8(30.8)	
Severe	0 (0)	0 (0)	
Disease severity skin n *(%)*			0.40
Quiescent	18(69.2)	19 (73.1)	
Mild	5 (19.2)	2 (7.7)	
Moderate	3 (11.5)	5 (19.2)	
Severe	0 (0)	0 (0)	
Physical activity level n *(%)*			0.69
Low	14 (53.8)	11 (42.3)	
Moderate	10 (38.5)	12 (46.2)	
High	2 (7.7)	3 (11.5)	
Medication type n *(%)*			0.73
Prednisone	14 (53.8)	13 (50)	
Prednisone+Methotrexate	5 (19.2)	3 (11.5)	
Prednisone+Azathioprine	2 (7.7)	4 (15.4)	
Prednisone +Mycophenolate mofetil	5 (19.2)	6 (23.1)	

Abbreviations: BMI, body mass index WC, waist circumference. [a]Variables are expressed as mean ± s.d. [b]*P*-values resulted from independent *t* tests for quantitative and Chi-square for qualitative variables between the two groups.

DISCUSSION

Results of current study revealed that administration of LC among subjects with PV for 8 weeks had favorable results on TAC, OSI and lipid profile; however, it did not significantly influence anthropometric indices, HDL-C and serum TOC concentration. This was the first research to study the effects of the LC supplementation on biomarkers of OS, antioxidant capacity and lipid profile, in PV patients.

The results of current study indicated that LC consumption could not decrease weight, BMI and WC significantly compared with the placebo. In agreement with the present trial, Malaguarnera *et al.*[22] indicated no significant reduction in BMI following the intake 2 g LC for 24 weeks in patients with non-alcoholic steatohepatitis. Moreover, several reports suggested that LC administration had no effect on weight reduction in subjects with type 2 diabetes,[23] osteoarthritis[24] or in obese women.[25] In contrast, Samimi *et al.*[26] reported that 250 mg/d LC for 12 weeks significantly affect body weight, BMI, WC and hip circumference in patients with PCOS. In addition, 4 g/d intravenous LC led to a substantial reduction in weight, BMI and WC in patients with metabolic syndrome.[7] Different study designs, different dosages of LC as well as duration of the study might have contributed to the ambiguity in the results that have been published.

The results of present trial indicated that administration of LC for 8 weeks in PV patients led to a significant reduction in serum TG, total- and LDL-C in comparison to the placebo. Moreover, within-group analysis showed a significant rise in HDL-C levels after LC supplementation. In consistent with our study, Spagnoli *et al.*[27] showed that receiving LC significantly reduced plasma TG, intermediate density lipoprotein (IDL) and VLDL-C in hyperlipidemic rabbits. Similar findings were also seen after the consumption of LC on lipid profiles in diabetic subjects.[10] Furthermore, results of a previous meta-analyses demonstrated that LC can significantly decrease the LDL-C in hemodialysis patients, however this meta-analysis has reported no advantageous effects on other lipid profiles, which is in contrast with our results.[28] In addition, it has to be pointed that some previous studies with different dosages of LC and interventional period did not found significant improvement in lipid profiles in patients with type 2 diabetes[23] and PCOS.[26] An exact mechanism through which LC intake might influence lipid profile is unknown. LC intake may change the liver metabolic bias apart from esterification and formation of TG into the synthesis of acetylcarnitines, which could increase fatty acids β-oxidation in mitochondria and reduce formation of TG and VLDL-C.[10]

Excessive production of ROS or insufficient scavenging of ROS lead to OS, which induces cellular damage, lipid peroxidation and

DNA impairment.[29] Some reports have indicated that PV is related to elevated ROS generation, OS and antioxidant depletion.[15,16,30] The present trial showed that LC intake in patients with PV was related to significant changes in serum TAC and OSI compared with placebo. In addition, within-group analysis indicated a significant decrease in TOC levels after LC supplementation. In

Table 2. The effect of L-carnitine supplementation on metabolic, oxidant and antioxidant parameters in patients with PV

Variable	*L-carnitine* (n = *26)*		*Placebo* (n = *26)*		P-*value*[a]
	mean ± s.d.	*Change*	*mean ± s.d.*	*Change*	
Weight (kg)					
Baseline	77.70 ± 13.25	− 0.63 ± 1.63	73.06 ± 9.32	0.10 ± 2.25	0.25
End of trial	77.06 ± 13.21		73.16 ± 9.27		
P-value[b]	0.058		0.81		
WC (cm)					
Baseline	94.85 ± 9.24	− 1.32 ± 3.76	90.87 ± 8.88	− 0.26 ± 1.73	0.35
End of trial	93.51 ± 8.77		90.59 ± 8.94		
P-value[b]	0.08		0.43		
BMI (kg/m^2)					
Baseline	28.07 ± 4.33	− 0.23 ± 0.60	26.66 ± 3.48	0.03 ± 0.93	0.29
End of trial	27.83 ± 4.29		26.70 ± 3.37		
P-value[b]	0.057		0.83		
Carnitine (nmol/ml)					
Baseline	73.61 ± 34.86	20.28 ± 16.94	77.12 ± 34.33	− 1.73 ± 5.58	< 0.001
End of trial	93.89 ± 40.58		75.38 ± 34.59		
P-value[b]	< 0.001		0.12		
TAC(µmol/l)					
Baseline	264.47 ± 25.16	13.24 ± 39.77	261.47 ± 25.99	− 14.78 ± 67.62	0.05
End of trial	277.72 ± 46.28		246.68 ± 63.84		
P-value[b]	0.10		0.27		
TOC (µmol/l)					
Baseline	9.53 ± 4.23	− 2.37 ± 4.48	7.72 ± 3.86	0.28 ± 4.52	0.15
End of trial	7.16 ± 4.41		8.00 ± 3.94		
P [b]	0.01		0.75		
OSI (a.u.)					
Baseline	3.59 ± 1.50	− 1.02 ± 1.61	2.98 ± 1.50	0.46 ± 1.94	0.01
End of trial	2.56 ± 1.47		3.44 ± 1.86		
P-value[b]	0.003		0.23		
TG(mg/dl)					
Baseline	142.30 ± 63.53	− 16.00 ± 21.71	137.65 ± 46.80	1.15 ± 29.28	0.02
End of trial	126.30 ± 55.66		138.80 ± 52.43		
P-value[b]	0.001		0.84		
TC(mg/dl)					
Baseline	213.84 ± 34.20	− 16.92 ± 24.13	204.61 ± 35.94	5.42 ± 17.66	˂0.001
End of trial	196.92 ± 23.51		210.03 ± 34.60		
P-value[b]	0.001		0.13		
LDL-C(mg/dl)					
Baseline	124.40 ± 24.66	− 15.41 ± 23.69	117.45 ± 33.01	0.26 ± 16.45	0.01
End of trial	108.99 ± 23.60		117.72 ± 30.73		
P-value[b]	0.003		0.93		
HDL-C(mg/dl)					
Baseline	58.11 ± 10.32	4.92 ± 4.59	59.76 ± 15.21	1.69 ± 7.04	0.06
End of trial	63.03 ± 11.05		61.46 ± 14.35		
P-value[b]	< 0.001		0.84		
VLDL-C (mg/dl)					
Baseline	29.66 ± 13.62	− 3.20 ± 4.34	29.04 ± 10.82	0.23 ± 5.85	0.02
End of trial	26.46 ± 12.50		27.27 ±11.77		
P-value[b]	0.001		0.84		

Abbreviations: ANCOVA, analysis of co-variance; BMI, body mass index; HDL-C, High density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OSI, oxidative stress index; TAC, total antioxidant capacity; TOC, total oxidant status; TG, triglycerides; TC, Total cholesterol; VLDL-C, Very Low density lipoprotein cholesterol; WC, waist circumference. [a]Obtained from ANCOVA, adjusted for baseline values. [b]Obtained from paired *T* test.

line with our study, lee *et al.* found that LC consumption resulted in significant rise in antioxidant enzymes activities and reduction in OS biomarkers in subjects with coronary artery disease.[18] Another study indicated that consumption of LC at a dose of 200 mg/kg/day significantly decreases OS in type 2 diabetic rats.[31] Furthermore, Fatouros *et al.*[32] reported that a 2-month LC intake could be beneficial in improving antioxidant status and reducing OS responses of subjects with end-stage renal disease. On the other hand, these finding contrast with a study that demonstrated that administration of LC for 8 weeks in osteoarthritis patients did not affect OS. In the present trial, absence of a significant effect of LC supplement on serum TOC concentration compared with placebo could be related to the insufficient duration of supplementation or different subjects of the study. The possible mechanisms by which LC affect OS may involve scavenging free radicals, interfering with the reactive oxygen species formation, facilitating the transmit of fatty acids into the mitochondria and decreasing mitochondrial superoxide production during hypoxia.[18,33] Previous reports have shown that excessive production of ROS and OS may play a substantial role in the pathophysiology and the development of immune-mediated disease, like PV and Behcet's, because ROS may change the structure and function of many molecules, like carbohydrates, lipids, DNA, and proteins, through glycoxidation and peroxidation.[15,34,35] Decrease in OS biomarkers and an increase in TAC levels after LC supplementation could be associated with the suppression of xanthine oxidase system from ROS generation.[36] Furthermore, LC consumption may decrease OS by chelating the metal ferrous ions and reducing the concentration of cytosolic iron.[33] In addition, LC supplementation affects OS through enhancing of antioxidants system components, such as glutathione peroxidase, vitamin A, E, and C.[14,37]

The strength of our trial was double-blind, randomized, placebo-controlled design and measuring the serum LC levels of the participants during the trial to evaluate the adherence to the LC supplementation. Also, the present study had some restrictions that need to be considered in interpreting the results. The study sample size was relatively small and the duration of intervention was short. Some non-significant changes may have become statistically significant with longer follow up. In addition, we were unable to measure the effect of LC supplementation on antioxidant enzymes and other biomarkers of OS in neutrophils and erythrocytes. This study was conducted on patients with PV who were taking four treatment regimens (prednisone, prednisone+methotrexate, prednisone+azathioprine and prednisone +mycophenolate mofetil). However, there were no difference in treatment regimen between two groups, our results should be interpreted with caution.

In conclusion, the current trial indicated that the intake of 2 g per day LC may have favorable results on TAC, OSI and lipid profiles in patients with PV. However, a null effect of LC on anthropometric indices and TOC and HDL-C in individuals with PV was documented in current study. These results support the concept that LC supplementation can be associated with positive effects on metabolic status and OS of patients with PV. However, further investigations are required to confirm these results.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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