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Effects of Alpha-Lipoic Acid Supplementation on Oxidative Stress Status in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial

Farshad Amirkhizi[1], Soudabeh Hamedi-Shahraki[2], Sonya Hosseinpour-Arjmand[1], Elnaz Vaghef-Mehrabany[1] and Mehrangiz Ebrahimi-Mameghani[3,*]

[1]Student Research Committee, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Department of Epidemiology and Biostatistics, Faculty of Public Health, Zabol University of Medical Sciences, Zabol, Iran
[3]Nutrition Research Center, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[*]*Corresponding author*: Nutrition Research Center, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Attar-Neishabouri Ave., Golgasht St., 516661471l, Tabriz, Iran. ebrahimimamagani@tbzmed.ac.ir

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Abstract

Background: Several mechanisms have been suggested to explain the pathogenesis of nonalcoholic fatty liver disease (NAFLD) and its progression, one of which is increased oxidative stress.
Objectives: This study aimed to evaluate the effect of alpha-lipoic acid (ALA) supplementation on anthropometric indices, dietary intake and oxidative stress-related parameters in obese patients with NAFLD.
Methods: In this double-blind, placebo-controlled trial, 50 NAFLD patients were assigned to two groups of receiving 1200 mg ALA (two 600 mg capsules of ALA) and placebo (two 600 mg capsules of placebo) for 12 weeks. Serum liver enzymes, malondialdehyde (MDA) level, total antioxidant status (TAS), and the activities of copper-zinc superoxide dismutase (Cu/Zn-SOD) and glutathione peroxidase (GSH-Px) were assessed at baseline and after 12 weeks of intervention.
Results: Serum concentrations of liver enzymes decreased significantly in the ALA group ($P < 0.05$ for all), while a noticeable decline was observed for alanine aminotransferase (ALT) in the placebo group (32.5 ± 18.9 vs. 25.9 ± 11.2; $P = 0.034$). Nonetheless, there were no significant differences between the study groups concerning serum liver enzymes concentrations post-intervention. Although ALA supplementation significantly reduced the serum concentration of MDA (2.52 ± 0.35 vs. 2.77 ± 0.49; $P < 0.040$) and increased serum TAS (1.73 ± 0.55 vs. 1.52 ± 0.34; $P < 0.048$), other oxidative stress-related parameters such as Cu/Zn-SOD and GSH-Px activities were not affected.
Conclusions: These findings suggest that daily supplementation of 1200 mg alpha-lipoic acid (ALA) for 12 weeks improves oxidative stress markers in patients with nonalcoholic fatty liver disease (NAFLD) and it could be considered as adjunctive therapy for the prevention of NAFLD progression.

Keywords: Alpha-Lipoic Acid, Non-Alcoholic Fatty Liver Disease, Obesity, Oxidative Stress

1. Background

Non-alcoholic fatty liver disease (NAFLD), is the most common liver disease associated with the increasing incidence of obesity and type 2 diabetes worldwide, it has a wide spectrum of liver manifestations ranging from simple liver steatosis to fibrosis and cirrhosis (1). Over the last two decades, NAFLD has become a major threat to public health (2), which is partly relevant to the global epidemic of obesity as one of the most important predictors of NAFLD (3).

Despite the dramatic rise in the prevalence of NAFLD, its pathogenesis has not been completely understood yet, and only a few beneficial therapies are available today. However, the increased generation of reactive oxygen species (ROS) caused by mitochondrial dysfunction plays a fundamental role in the progress of NAFLD (4). The adverse effects of ROS on hepatocytes are hindered by protective mechanisms consisting of enzymatic (e.g., superoxide dismutase and glutathione peroxidase) and non-enzymatic antioxidants (5).

Because of the multifactorial nature of NAFLD, it is implausible that one single treatment represents a panacea for this disease. One of the current strategies to achieve optimal management of NAFLD progression, especially in

obese patients, is lifestyle modifications such as stable weight loss through following a low-calorie diet and increased physical activity (6-8). Considering the role that oxidative stress serves in the pathogenesis of NAFLD, antioxidant therapy combined with lifestyle modifications and medications (e.g., insulin sensitizers and lipid-lowering drugs) appears to be an effective therapeutic strategy to prevent the progression of the disease.

Alpha-lipoic acid (1, 2-dithiolane-3-pentanoic acid) (ALA), a strong natural antioxidant containing sulfhydryl groups, is an essential cofactor for several enzymes involved in energy metabolism and may also improve mitochondrial function (9). Current evidence indicates that ALA is a potent antioxidant that not only acts as an antioxidant in the cytosol, but also has antioxidant activity in cell membranes, a characteristic discriminating it from other antioxidants (10, 11). Furthermore, the important indirect role of ALA in the regeneration of other antioxidants such as glutathione and Vitamins C and E has also been shown (12, 13). In a rat model, Lykkesfeldt et al. showed that ALA feeding increased hepatic ascorbate level in rats (14). The solubility of this small antioxidant molecule in aqueous and lipid environments makes it effective against a wide range of free radicals (15). Recently, it has also been demonstrated that ALA could prevent hepatic steatosis in rats receiving a high-fat diet (16). In addition to its antioxidant effects, ALA has been shown to have anti-obesity properties (17, 18). Although some studies have already illustrated beneficial effects of ALA on some disease (17, 19), to the best of our knowledge, no trials have been conducted to investigate the effects of ALA supplementation on NAFLD patients.

2. Objectives

As ALA may have a protective effect against the progression of NAFLD through reduction of oxidative stress, this study sought to examine the effect of ALA supplementation on oxidative stress biomarkers including serum malondialdehyde (MDA) level, serum total antioxidant status (TAS), and copper-zinc superoxide dismutase (Cu/Zn-SOD) and glutathione peroxidase (GSH-Px) activities in patients with NAFLD.

3. Methods

3.1. Participants

The subjects included in this study were obese patients newly diagnosed with NAFLD; they aged 20 - 50 years old and had a body mass index (BMI) of 30 - 40 kg/m^2. In total, 136 subjects were enrolled in the study. Of these, 86 subjects were excluded (64 subjects did not meet the inclusion criteria, 17 subjects refused to participate, and five subjects for other reasons), and 50 patients were included in the randomization process (Figure 1). These patients were recruited from among those referred to Sheykholrayis Polyclinic affiliated to Tabriz University of Medical Sciences, Tabriz, Iran, from April 2016 to September 2016. In addition to referral by a gastroenterologist, public printed advertisements were also used to recruit more patients.

Ultrasonography technique and elevated liver enzymes were used for the diagnosis of NAFLD. Echogenicity grading of the liver was performed according to Saverymuttu et al. (20). A single expert sonographer conducted all the procedures using a SonoAce X4 ultrasound system (South Korea).

The exclusion criteria were any history of alcohol and/or drug abuse, tobacco use, chemotherapy during the previous year, history of cirrhosis, viral or autoimmune hepatitis, drug-induced hepatic disorders or any other liver diseases, Wilson's disease, hemochromatosis, kidney dysfunctions, diabetes, rheumatoid arthritis, or malignant tumors, antioxidant (except for Vitamin E), Omega-3 supplements consumption for at least six months prior to participation in our study or throughout the study, use of antihypertensives, lipid-lowering medications, contraceptives or estrogen, pregnancy or breastfeeding, and unwillingness to continue the study.

First, all the participants were given a full explanation about the objectives and protocol of the study, and then written informed consent was obtained from all the participants before the commencement of the trial. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, and it was registered in Iranian Registry of Clinical Trials' website (available at http://www.irct.ir, registration ID: IRCT201511143320N12).

3.2. Study Design

The study was designed as a randomized, double - blind, placebo-controlled clinical trial with two parallel groups. Block randomization of size four was used for random allocation of the subjects into study groups. For allocation of the subjects, Random Allocation Software (RAS) was used for computer-generated random sequences. Using stratified randomized selection for age, sex, and BMI, the subjects were randomly allocated to the ALA (n = 25) and placebo (n = 25) groups. Subjects in the ALA group received two capsules, each containing 600 mg ALA (i.e., 1200 mg/day) plus 400 mg Vitamin E per day, while those in the placebo group received two placebo capsules (containing starch) plus 400 mg Vitamin E daily for 12 weeks. The

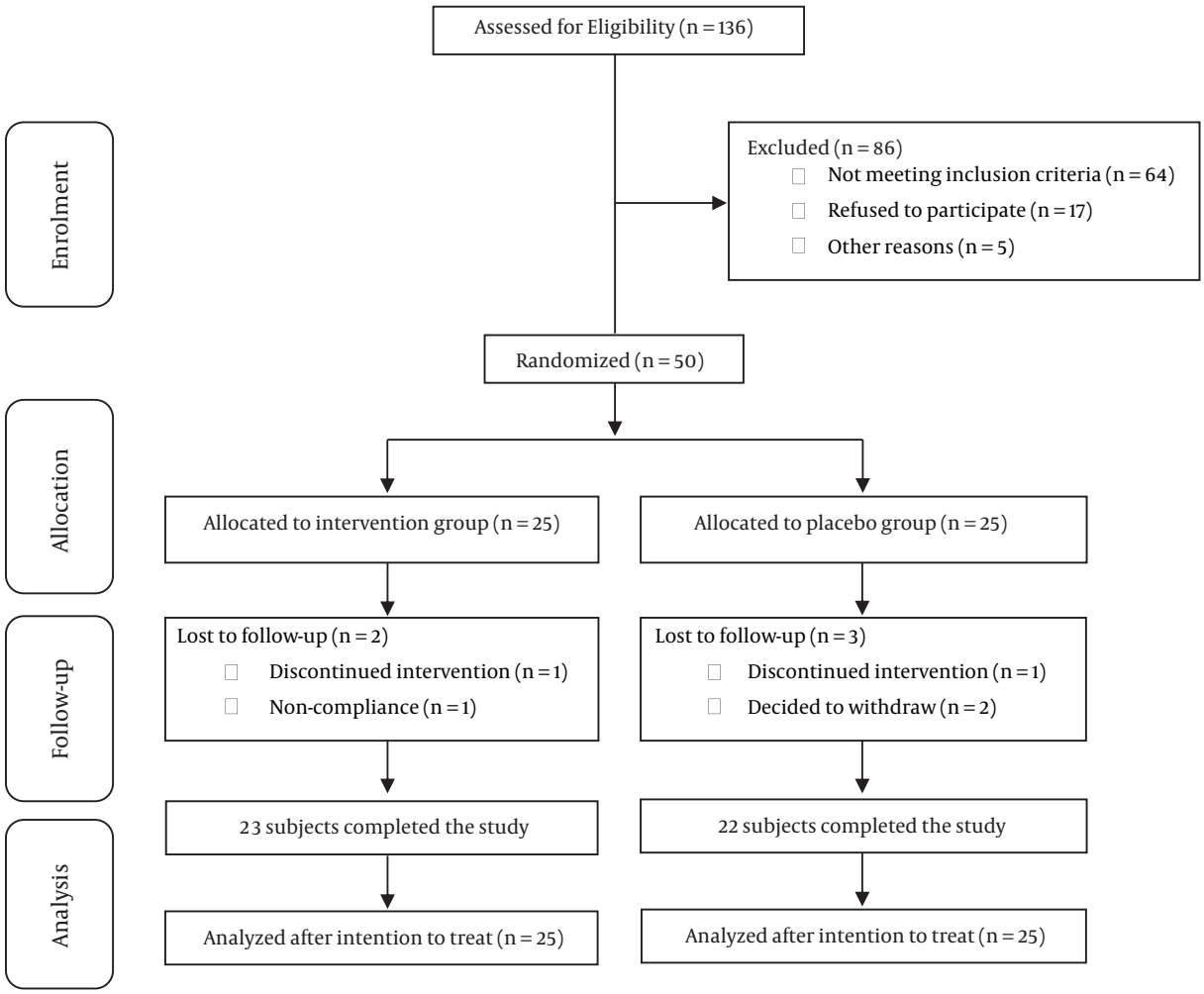

Figure 1. Flow diagram of the study

subjects were advised to take ALA or placebo capsules at an interval of 12 hours with breakfast and dinner. ALA and placebo capsules were identical in shape, color, odor, and volume and were handed over every three weeks. The subjects were followed by telephone calls each week to obtain the relevant information. Furthermore, at baseline, the subjects received consultation on a healthy diet for NAFLD.

On each visit, the subjects were also asked whether they had experienced any side effects during the medication administration. To avoid any bias, the study subjects and those who involved in enrolling, treating and assessing the subjects were blinded to study assignment, and capsules (ALA and placebo) were packaged and coded by a third person who had no involvement in the study.

3.3. Sample Size Calculation

On the basis of the study by McNeilly et al. (21), the SD of serum TAS was 0.35 mmol/L and the difference in mean (d) of TAS level was 0.3 mmol/L. Considering type 1 (α) and type 2 errors of 0.05 and 0.2 (power = 80%), respectively, a sample size of 21 patients was determined in each group using the following formula:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 \times \left(SD_1^2 + SD_2^2\right)}{d^2} \quad (1)$$

Sample size was increased to 25 subjects in each study group considering a probable dropout of 10%.

3.4. Anthropometric Measurements

Body weight was measured to the accuracy of 100 g, while subjects had light clothes and no shoes on, using a

Seca scale (Hamburg, Germany). Seca body meter (Hamburg, Germany) was used to measure height in standing position with 0.5 cm accuracy while subjects were not wearing shoes. BMI was then calculated via dividing weight (in kilogram) by the square of height (in meters). To calculate the waist-to-hip ratio (WHR), the waist circumference (WC) was measured at the narrowest level to the nearest 0.1 cm, and hip circumference (HC) was measured at the maximum level to the nearest 0.1 cm. To decrease error, all the measurements at the beginning and end of the study were made by the same person.

3.5. Dietary and Physical Activity Assessment

Dietary intakes of the subjects were assessed by three 24-hour food recalls at baseline and the end of the study to ensure they maintained their usual diet throughout the intervention. Food intakes were then converted to energy and other nutrients using Nutritionist-IV (N4) software (N-Squared Computing, Cincinnati, OH, USA) modified for Iranian foods.

Physical activity was also evaluated using the metabolic equivalent of task (MET) questionnaire (22) at the beginning and end of the study.

3.6. Blood Sampling and Laboratory Measurements

Venous blood samples (10 mL) were obtained from the median cubital vein after a 12-hour overnight fasting at baseline and after 12 weeks of intervention. For separating serum, whole blood samples were centrifuged for 15 minutes at 3000 rpm, and the collected serum samples were immediately stored at -80°C until the assays. In order to assay the Cu/Zn-SOD and GSH-Px activities, whole blood samples were rinsed three times with cold saline (9.0 g/L NaCl) and hemolyzed by addition of an equal volume of ice-cold demineralized ultrapure water. Subjects' hemolysates were also stored at -80°C until analysis.

In order to express the enzyme activities per gram of hemoglobin (Hb), Hb concentration was measured in the hemolysates by a standard kit (Zist Chemistry Laboratories, Iran) involving the cyanmethemoglobin method (Drabkin's method). The activity of Cu/Zn-SOD (EC 1.15.1.1) was measured by Ransod kit (Randox Laboratories, Ltd., UK, cat. no.SD-125). Measurement of GSH-Px (EC 1.11.1.9) activity was performed by Ransel kit (Randox Laboratories, Ltd., UK, cat.no. RS-504). Serum TAS was determined using Randox TAS kit (Randox Laboratories, Ltd., UK, cat.no. NX-2332). The serum MDA level was also assessed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Uchihara and Mihara's method (23). Serum concentrations of liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assessed using Abbott ALCYON 300 auto-analyzer kits (Pars-Azmoon, Tehran, Iran).

3.7. Statistical Analysis

The statistical analyses were performed using SPSS Statistic Software for Windows, version 16.0 (SPSS Inc., Chicago, ILL., USA), and the descriptive data were expressed as mean ± standard deviation (SD) for continuous variables and number (percentage) for categorical variables. Final analysis of the data was performed based on intention-to-treat approach.

To check the normal distribution of the variables, we applied the Kolmogorov-Smirnov criterion. Based on the results of this test, all quantitative variables in this study had normal distributions. To compare continuous variables intra- and inter-groups, paired t-test, and independent t-test were used, respectively. In order to omit the effects of confounding factors, analysis of covariance (ANCOVA) was used to identify any differences in continuous variables between groups' post-intervention, adjusting for baseline measurements and confounding factors (i.e., BMI, energy intake, and physical activity changes throughout the study). The P value less than 0.05 was considered statistically significant.

4. Results

4.1. Subject Characteristics

Totally, 50 obese patients with NAFLD were enrolled in the study and underwent randomization (Figure 1), 25 patients were assigned to ALA group and 25 patients were assigned to placebo group. In the ALA group, two subjects withdrew from the study because of discontinued intervention (n = 1) and poor compliance (n = 1). In the placebo group, there were also three dropouts because of discontinued intervention (n = 1) and withdrawal for personal reasons (n = 2). Finally, 22 subjects in the ALA group and 23 subjects in the placebo group completed the trial. However, as the analysis was based on an intention-to-treat approach, all the 50 subjects (25 in each group) were included in the final analyses. The compliance rate of the patients based on the percentage of the taken capsules by the patients throughout the trial was about 98% and 96% in the ALA group and placebo group, respectively. None of the participants reported adverse effects or symptoms throughout the trial period.

The baseline characteristics of the subjects in the study groups are provided in Table 1. The mean ages in the ALA and placebo groups were 40.28 ± 5.50 and 37.52 ± 9.67 years, respectively. Furthermore, 52.0% and 56.0% of the

subjects in the ALA and placebo groups were male, respectively. As presented in Table 1, 36% of the subjects in the ALA group and 12% in the placebo groups had severe liver steatosis (grade 3).

4.2. Dietary Intake, Anthropometric Indices, and Liver Enzymes Measures

At the end of the study, compared to baseline values, a significant decrease in mean energy and carbohydrate intakes was observed in both groups, while protein intake decreased only in the placebo group ($P < 0.05$ for all). However, there were no significant differences in energy and macronutrient intakes between the study groups neither at baseline nor at the end of the study. Furthermore, physical activity level did not change significantly within and between the two groups throughout the study period ($P > 0.05$) (data not shown).

Baseline values of weight, BMI, WC, HC, and WHR were not significantly different between the study groups (Table 2). Despite significant alleviations in the mentioned variables ($P < 0.05$ for all), except for WHR in both groups throughout the study, no noticeable differences were observed between the groups in these variables at the end of the study.

The effect of ALA and placebo supplementation on serum levels of liver enzymes (i.e., ALT, AST, and ALP) is shown in Table 2. After intervention, compared to the outset of the study, a significant reduction in serum levels of liver enzymes (i.e., ALT, AST, and ALP) was observed in the ALA group ($P < 0.05$ for all); however, in the placebo group, a significant decline was observed only in serum ALT concentration ($P = 0.034$). Furthermore, AST/ALT ratio values remained unchanged in both groups at the end of the study compared to baseline. There were no significant differences between the two groups in terms of serum liver enzymes levels at the end of the study.

4.3. Oxidative Stress Related-Parameters

The comparison of the mean changes of oxidative stress related-parameters within and between groups is presented in Table 3. Serum level of MDA decreased significantly after the intervention in the ALA group ($P = 0.016$), while no changes in the placebo group were observed. Conversely, ALA consumption caused a significant increase in serum TAS ($P = 0.031$) compared to the baseline values, while no significant changes were found in the placebo group. Compared to the placebo group, subjects who received ALA showed a significant change in serum MDA level ($P = 0.040$). A marginally significant change in serum TAS was also noted between the two groups at the end of our study ($P = 0.048$). Regarding Cu/Zn-SOD and GSH-Px, no significant changes were observed within or between the two groups at baseline and after the intervention.

5. Discussion

The results of this study revealed that daily supplementation of 1200 mg ALA for 12 weeks does not have any significant effects on dietary intake, anthropometric indices, and liver enzymes in obese patients with NAFLD. Moreover, ALA supplementation along with lifestyle modifications resulted in improved serum TAS and a significant reduction in serum MDA level, but it did not affect Cu/Zn-SOD and GSH-Px antioxidant enzymes activities. Although the beneficial effects of ALA on some diseases, anthropometric indices (17, 24, 25), and oxidative status (26-28) have been previously studied, to the best of our knowledge, no trials have been conducted to investigate the effects of ALA supplementation on NAFLD characteristics including oxidative stress status.

The results of this study indicated that ALA supplementation does not significantly affect anthropometric indices (i.e., body weight, BMI, WC, and HC) and dietary intake. Numerous studies have examined the effect of ALA on weight changes. For instance, in contrast to the findings of the current study, Kim et al. (24) reported that body weight and BMI remarkably reduced following daily consumption of 1200 mg ALA for 12 weeks in schizophrenic patients. In another study, daily supplementation of 600 mg ALA for 12 weeks decreased body weight, BMI, and waist circumference in chronic spinal cord patients (25). It must be noted that participants in the mentioned studies were subjects diagnosed with neurological disorders, while in our study, subjects were NAFLD patients; therefore, these contrasting results may be partly due to the different features of these disorders and the ALA dose consumed. In accordance with our finding, Koh et al. (17) also observed no changes in body weight after 20 weeks of 1200 mg/day ALA supplementation in 360 obese subjects.

It has been established that high production of reactive oxygen species (ROS) plays an important role in the pathogenesis and development of NAFLD (29, 30). In this study, we assessed the effect of ALA supplementation on serum concentration of MDA, one of the products of lipid peroxidation, and activities of Cu/Zn-SOD and GSH-Px, two major enzymes affording antioxidant protection. The cumulative action of all known and unknown antioxidants present in serum was also assessed by measuring serum TAS.

Although some investigators have examined the effect of ALA on oxidative stress markers in diabetic patients (28),

Table 1. Baseline Characteristics of Patients with Non-Alcoholic Fatty Liver Disease (N = 25)[a]

Characteristic	ALA Group	Placebo Group	P Value
Age, y	40.28 ± 5.50	37.52 ± 9.67	0.318[b]
Sex			0.777[c]
Male	13 (52.0)	14 (56.0)	
Female	12 (48.0)	11 (44.0)	
Marital status			0.123[c]
Single	2 (8.0)	6 (24.0)	
Married	23 (92.0)	19 (76.0)	
Educational level			0.333[c]
Primary	5 (20.0)	8 (32.0)	
Diploma and higher	20 (80.0)	17 (68.0)	
Physical activity, MET-h/day	25.7 ± 6.4	29.1 ± 7.2	0.678[b]
Steatosis grade			0.072[c]
Grade 1	5 (20.0)	11 (44.0)	
Grade 2	11 (44.0)	11 (44.0)	
Grade 3	9 (36.0)	3 (12.0)	

Abbreviations: ALA, alpha-lipoic acid; MET, metabolic equivalent of task.
[a]Values are expressed as No. (%) or mean ± SD.
[b]P values obtained from independent sample *t*-test.
[c]P values obtained from Pearson's Chi-squared test.

patients with multiple sclerosis (MS) (27), and animal models (31, 32), data for NAFLD patients is lacking. In the previous studies, increased free radicals generation was reported in NAFLD patients (33-35). In the present study, ALA supplementation significantly decreased serum MDA level. Similar to our findings, Stankovic et al. (36) reported a decrease in chronic oxidative stress in an animal model of NAFLD induced by methionine-choline- deficient diet. Another study (37) also reported that ALA had a noticeable effect on MDA level in old rats. However, other studies have not reached such findings (27, 38).

Two possible factors may lead to elevated lipid peroxidation including an increase in free radicals generation and the decrease in enzymatic and non-enzymatic defense system. After confirmation of the beneficial effects of ALA supplementation on serum MDA level (as an oxidative stress marker), we attempted to identify the factors associated with reduced MDA level. Therefore, we examined the activities of antioxidant enzymes (i.e., Cu/Zn-SOD and GSH-Px) and serum TAS in our participants.

Our findings showed that 12-week ALA supplementation in patients with NAFLD changed neither Cu/Zn-SOD nor GSH-Px activities. Cu/Zn-SOD, the first line of antioxidant defense, converts superoxide anions into oxygen and H_2O_2. Then, H_2O_2 is detoxified by other antioxidant enzymes such as GSH-Px and catalase. In a randomized cross-over design study, Sharman et al. (39) could not find any significant changes in Cu/Zn-SOD activity following oral supplementation with 600 mg ALA once daily in healthy elderly men. Another study showed that in patients with MS, 12 weeks of oral supplementation with 1200 mg/day ALA did not significantly affect Cu/Zn-SOD activity (27). Moreover, Freitas et al. (40) failed to show the effect of ALA consumption on Cu/Zn-SOD activity in seizures of rats. Inversely, Arambasic et al. (41) reported increased Cu/Zn-SOD activity in renal tissue of ALA-treated diabetic rats after four weeks of intervention. Furthermore, intraperitoneal administration of ALA has been shown to have remarkable effects on increasing Cu/Zn-SOD activity in various brain regions of aged rats (42).

The discrepancy observed between our results and those of the animal studies mentioned (41, 42) might be attributable to the different physiological mechanisms between animals and humans in ALA metabolism and redox system regulation. In addition, these contentious results may be related to differences in ALA dosage, duration of the supplementation, and especially the severity and type of the disease investigated.

Regarding GSH-Px activity, no significant changes were observed within and between the groups by the end of our study. This finding was consistent with those of Sharman et al. (39) and Khalili et al. (27), who reported that ALA ad-

Table 2. Anthropometric Indices and Liver Enzymes Measures of Patients with Non-Alcoholic Fatty Liver Disease Throughout the Study (N = 25)[a,b]

Variables	ALA Group	Placebo Group	MD (CI 95%)	P Value
Weight, kg				
Baseline	92.1 ± 10.7	93.7 ± 15.5	1.5 (-6.3, 9.4)	0.694[c]
After 12 weeks	88.7 ± 10.7	90.3 ± 14.3	0.3 (-1.2, 1.7)	0.704[d]
MD (CI 95%)	-3.5 (-4.5, -2.4)	-3.4 (-4.8, -2.0)		
P value[e]	< 0.0001	< 0.0001		
BMI, kg/m^2				
Baseline	33.6 ± 3.7	34.1 ± 4.5	0.5 (-1.9, 2.9)	0.677[c]
After 12 weeks	32.3 ± 3.6	32.9 ± 4.2	0.2 (-0.4, 0.7)	0.578[d]
MD (CI 95%)	-1.3 (-1.7, -0.9)	-1.2 (-1.7, -0.7)		
P value[e]	< 0.0001	< 0.0001		
WC, cm				
Baseline	112.0 ± 24.8	107.7 ± 10.1	-4.3 (-15.7, 7.2)	0.456[c]
After 12 weeks	109.6 ± 23.8	105.5 ± 10.9	0.4 (-1.6, 2.4)	0.699[d]
MD (CI 95%)	-2.4 (-3.2, -1.6)	-2.2 (-4.3, -0.2)		
P value[e]	< 0.0001	0.032		
HC, cm				
Baseline	117.3 ± 24.2	118.5 ± 24.9	1.2 (-13.3, 15.8)	0.863[c]
After 12 weeks	114.5 ± 24.2	116.4 ± 25.1	0.7 (-0.6, 2.1)	0.265[d]
MD (CI 95%)	-2.7 (-3.6, -1.9)	-2.1 (-3.1, -1.0)		
P value[e]	< 0.0001	0.001		
WHR				
Baseline	0.97 ± 0.23	0.93 ± 0.11	-0.05 (-0.15, 0.06)	0.392[c]
After 12 weeks	0.98 ± 0.23	0.92 ± 0.10	-0.01(-0.02, 0.01)	0.649[d]
MD (CI 95%)	0.01 (-0.01, 0.01)	0.01 (-0.02, 0.01)		
P value[e]	0.459	0.589		
ALT, U/L				
Baseline	37.0 ± 25.0	32.5 ± 18.9	-7.0 (-23.3, 9.3)	0.391[c]
After 12 weeks	29.0 ± 20.3	25.9 ± 11.2	-1.6 (-8.8, 5.7)	0.664[d]
MD (CI 95%)	-8.1 (-15.4, -0.8)	-6.7 (-12.8, -0.5)		
P value[e]	0.032	0.034		
AST, U/L				
Baseline	26.1 ± 15.7	24.2 ± 10.6	-1.9 (-10.0, 6.1)	0.637[c]
After 12 weeks	19.8 ± 10.2	20.9 ± 5.7	1.7 (-2.3, 5.6)	0.401[d]
MD (CI 95%)	-6.2 (-11.2, -1.3)	-3.2 (-7.0, 0.5)		
P value[e]	0.015	0.089		
AST/ALT ratio				
Baseline	0.77 ± 0.26	0.88 ± 0.38	0.11 (-0.09, 0.30)	0.279[c]
After 12 weeks	0.78 ± 0.26	0.92 ± 0.35	0.1 (-0.1, 0.2)	0.210[d]
MD (CI 95%)	0.01 (-0.09, 0.12)	0.05 (-0.09, 0.18)		
P value[e]	0.803	0.476		
ALP, U/L				
Baseline	211.5 ± 57.4	213.1 ± 60.9	1.6 (-33.5, 36.9)	0.926[c]
After 12 weeks	201.1 ± 64.1	202.7 ± 49.1	1.0 (-13.4, 15.4)	0.888[d]
MD (CI 95%)	-10.4 (-20.7, -0.1)	-10.4 (-21.0, 0.1)		
P value[e]	0.048	0.052		

Abbreviations: ALA, alpha-lipoic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HC, hip circumference; MD, mean differences; WC, waist circumference; WHR, waist-to-hip ratio.
[a]Values are expressed as mean ± SD.
[b]P < 0.05 was considered significant.
[c]P values obtained from independent sample *t*-test.
[d]P values obtained from ANCOVA; adjusted for baseline values, energy intake and physical activity changes.
[e]P values obtained from paired *t*-test.

Table 3. Oxidative Stress-Related Biomarkers Measures of Patients with Non-Alcoholic Fatty Liver Disease Throughout the Study (N = 25) [a,b]

Variables	ALA Group	Placebo Group	MD (CI 95%)	P Value
MDA, nmol/mL				
Baseline	2.71 ± 0.45	2.75 ± 0.41	0.04 (-0.22, 0.30)	0.744[c]
After 12 weeks	2.52 ± 0.35	2.77 ± 0.49	0.23 (0.01, 0.44)	0.040[d]
MD (CI 95%)	-0.19 (-0.34, -0.04)	0.01 (-0.19, 0.22)		
P value[e]	0.016	0.892		
Cu/Zn-SOD, U/g Hb				
Baseline	1288.5 ± 167.5	1279.1 ± 175.5	-9.4 (-111.3, 95.5)	0.853[c]
After 12 weeks	1250.3 ± 133.7	1297.4 ± 164.5	52.1 (-20.3, 124.4)	0.154[d]
MD (CI 95%)	-38.2 (-79.0, 2.7)	18.3 (-61.1, 97.7)		
P value[e]	0.066	0.637		
GSH-Px, U/g Hb				
Baseline	39.4 ± 13.2	45.7 ± 10.5	6.3 (-0.8, 13.4)	0.082[c]
After 12 weeks	37.3 ± 12.4	46.9 ± 11.1	5.4 (-0.1, 10.9)	0.054[d]
MD (CI 95%)	-2.1 (-6.3, 2.1)	1.2 (-2.7, 5.2)		
P value[e]	0.309	0.529		
TAS, mmol/L				
Baseline	1.50 ± 0.48	1.50 ± 0.36	0.01 (-0.25, 0.27)	0.926[c]
After 12 weeks	1.73 ± 0.55	1.52 ± 0.34	-0.22 (-0.44, -0.01)	0.048[d]
MD (CI 95%)	0.23 (0.02, 0.45)	0.02 (-0.06, 0.10)		
P value[e]	0.031	0.602		

Abbreviations: ALA, alpha-lipoic acid; Cu/Zn-SOD, copper-zinc superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; TAS, total antioxidant status.
[a] Values are expressed as mean ± SD.
[b] $P < 0.05$ was considered significant.
[c] P values obtained from independent sample *t*-test.
[d] P values obtained from ANCOVA; adjusted for baseline values, body mass index, energy intake and physical activity changes.
[e] P values obtained from paired *t*-test.

ministration does not affect GSH-Px activity. However, non-significant difference in GSH-Px activity in patients with NAFLD as compared to controls has been reported previously (34). This may be the reason for the ineffectiveness of ALA supplementation on GSH-Px activity.

Even though we did not find beneficial effects of ALA on antioxidant enzymes activities, we found benefits in terms of the non-enzymatic antioxidant defense system based on serum TAS. This may be attributed to the decline in the serum level of MDA observed in our study. In other words, beneficial effects of ALA supplementation on improved serum MDA level might result from its effect on the non-enzymatic antioxidant defense system. In a similar study by Martins et al. (43) a significant increase in serum total antioxidant capacity (TAC) was seen with daily supplementation (200 mg/day) of ALA in sickle cell patients. Another study showed that in patients with MS, 12 weeks of oral supplementation with 1200 mg/day ALA did not significantly affect serum TAC (27). In contrast to our results, daily supplementation of ALA (600 mg/day) for 16 weeks did not change TAC in non-obese and non-diabetic polycystic ovary syndrome subjects (44). Discrepant findings might be explained by different laboratory methods used, diversity in subjects' conditions, different dosages of ALA supplementation, and varied duration of supplementation. Several mechanisms may explain the effects of ALA supplementation on increased serum TAS and consequently, reduced MDA. Some evidence suggests that ALA exerts its antioxidant activity through either directly scavenging ROS (13) or recycling endogenous antioxidants such as glutathione (12), coenzyme Q_{10}, and Vitamins C and E (45). Furthermore, ALA may have an indirect effect on oxidative stress mediated by chelating redox-active transition metals such as iron and copper (46).

The several limitations of the present trial must be taken into account in the interpretation of our find-

ings. Diagnosis of NAFLD was only based on ultrasonography results, making diagnosis somewhat subjective and operator-dependent. Furthermore, owing to limited funding, we could not assess other oxidative stress-related biomarkers such as catalase and glutathione reductase activities and blood glutathione levels. Finally, funding restraints did not allow for measurement of serum ALA concentration. However, this limitation was controlled to some extent by repeated follow-up visits and counting the remaining capsules.

This study also had several strengths including a relatively high compliance rate of the participants (more than 95%) in both groups, a low drop-out rate, narrow BMI range of the patients, adequate study duration, and double-blind, placebo-controlled design.

5.1. Conclusion

In sum, the results of this clinical trial suggest that 1200 mg/day of ALA supplementation had beneficial effects on some of the oxidative stress-related parameters including serum MDA and TAS levels and may be effective in preventing NAFLD progression.

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Footnotes

Authors' Contribution: Farshad Amirkhizi carried out the design of the study and participated in data analysis, measurement of biochemical values, delivery of interventions and prepared the first draft of the manuscript. Soudabeh Hamedi-Shahraki provided assistance in the design of the study, coordinated in prepared the manuscript and did the statistical analyses. Sonya Hosseinpour-Arjmand provided assistance in the measurement of biochemical values, gathering data especially anthropometric and dietary intake data and coordinate in the delivery of interventions. Elnaz Vaghef-Mehrabany provided assistance in the design of the study and edited the manuscript. Mehrangiz Ebrahimi-Mameghani provided assistance in the design of the study, supervised data collection and edited the manuscript.

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