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The Effect of Alpha-Lipoic Acid on Liver Function and Metabolic Markers in Obese Patients with Non-Alcoholic Fatty Liver Disease: A Double - Blind Randomized Controlled Trial

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Abstract

Background: Insulin resistance has a pivotal role in the occurrence of impaired glucose tolerance and dyslipidemia in patients with Non-Alcoholic Fatty Liver Disease (NAFLD). There is evidence of possible beneficial effects of Alpha-Lipoic Acid (ALA) on insulin resistance and metabolic disorders.

Objectives: This study aimed at examining the effects of ALA supplementation on liver enzymes, insulin sensitivity, glucose markers, and lipid profile in obese patients with NAFLD.

Methods: In this double-blind placebo-controlled randomized clinical trial, 50 obese patients with NAFLD were randomly allocated to "ALA group" (received 1200 mg ALA as two capsules per day) or "Placebo group" (received placebo containing cornstarch as two capsules per day) for 12 weeks. Anthropometric measures, dietary intakes, liver enzymes as well as glucose markers and lipid profile were assessed at baseline and after 12 weeks of intervention.

Results: Forty-five patients completed the study (ALA group = 23; placebo group = 22). Liver enzymes were not significantly altered by the intervention group. Alpha Lipoic Acid supplementation led to a significant attenuation in serum levels of insulin (13.4 $\pm$ 5.4 vs. 18.1 $\pm$ 8.6; P = 0.019) and triglyceride (146.9 $\pm$ 60.6 vs. 186.3 $\pm$ 54.2; P = 0.037) in comparison with the placebo group, yet did not affect other lipid profile parameters, Fasting Serum Glucose (FSG) and β-cell function index (HOMA-B) in patients with NAFLD. furthermore, quantitative insulin sensitivity check index (QUICKI) increased significantly in the ALA group compared to the placebo (0.329 $\pm$ 0.025 versus 0.317 $\pm$ 0.020; P = 0.033).

Conclusions: Patients with NAFLD may benefit from ALA supplementation, at least partially through augmented insulin sensitivity and improvement of lipid profile.

Keywords: Alpha-Lipoic Acid, Dyslipidemia, Fatty Liver, Glucose, Insulin Resistance, Non-Alcoholic, Obesity

1. Background

The obesity epidemic has tremendously grown into a major public health challenge worldwide during the last decades (1). Similarly, nonalcoholic fatty liver disease (NAFLD) has become one of the chief diseases around the globe (2). Furthermore, NAFLD, the hepatic sign of metabolic syndrome, is the leading cause of chronic liver diseases with a wide - spectrum of liver damage ranging from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), hepatic cirrhosis, and ultimately hepatocellular carcinoma (3).

Although current attempts to clarify the mechanisms and pathogenesis of NAFLD have answered some questions, further investigations are still warranted. Insulin resistance (IR), as a major factor in marked hepatic fat accumulation, has a pivotal role in the occurrence of impaired glucose tolerance and dyslipidemia in patients with NAFLD (4).

The adverse effect of NAFLD on the risk of cardiovascular diseases (CVD) has gained intense scientific interest in the past decade. In the majority of patients, NAFLD is closely associated with insulin resistance, dyslipidemia, and hyperglycemia and being overweight or obese, which

are all established risk factors for CVD (5). The most common form of dyslipidemia in patients with NAFLD is atherogenic dyslipidemia, which is characterized by hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) levels, and high low-density lipoprotein cholesterol (LDL-C) levels (6).

To the best of the author's knowledge, there are no established effective pharmacologic treatments for NAFLD and only lifestyle modifications, such as weight reduction, are considered helpful for the management of the disease (7). However, complementary therapies and using nutraceutical supplements with lipid-lowering and insulin-sensitizing properties seem to be a good strategy for improvement of NAFLD characteristics.

Alpha-lipoic acid (ALA), a nutraceutical supplement produced in small quantities from octanoic acid in the mitochondria, functions as a cofactor in mitochondrial α-ketoacid dehydrogenases. Although numerous studies indicate that ALA improves insulin sensitivity (8, 9), it remains questionable whether ALA supplementation effectively improves CVD risk factors in patients with NAFLD. Park et al. (10) showed that ALA feeding in rats reduces hepatic lipogenesis by activating AMP-Activated Protein Kinase (AMPK) and suppressing sterol regulatory binding protein-1c (SREBP-1c) activity. It has also been recently revealed that ALA has the ability to ameliorate hypertriglyceridemia by preventing both synthesis of triacylglycerol, and secretion of very low-density lipoprotein (VLDL) triacylglycerol in Zucker fatty rats afflicted with diabetes (11). Although the beneficial effects of ALA in some diseases have been previously studied (8, 12), to the best of the author's knowledge, this was the first human study to examine the effects of ALA supplementation on glycemic biomarkers, liver enzymes, and lipid profile in NAFLD. Therefore, the researchers designed this randomized clinical trial to assess the effects of ALA supplementation on liver enzymes, insulin sensitivity, glucose markers, and lipid profile in obese patients with NAFLD.

2. Methods

2.1. Subjects

This clinical trial was carried out on 50 obese patients, who were newly diagnosed with NAFLD referred to governmental Sheykholrayis polyclinic in Tabriz, Iran from April 2016 to September 2016. The recruitment of patients was done through public printed advertisements and referral by a gastroenterologist. Prescreening of the volunteers was performed by phone interviews, and the subjects, who met the initial inclusion and exclusion criteria, were screened with respect to the study inclusion criteria on the first visit. All participants underwent ultrasonography scanning for fatty liver diagnosis by a single ultrasonographist, using a SonoAce X4 ultrasound system (South Korea). Non - alcoholic fatty liver disease is defined by the presence of steatosis in liver (as detected by ultrasonography), according to Saverymuttu et al. (13). Based on echogenicity, beam penetration, and portal vessel wall distinction, nonalcoholic fatty livers were classified into three subscale grades (grade 1, 2 and 3).

The inclusion criteria were a diagnosis of hepatic steatosis on the basis of characteristic ultrasonography features, having a body mass index (BMI) ranging from 30 to 40 kg/m^2, age between 20 and 50 years for both genders, and willingness to participate in the study. The exclusion criteria included pregnancy or lactation, hormone therapy or taking oral contraceptives, chemotherapy during the previous year, history of cardiovascular diseases, hypertension, diabetes mellitus, thyroid disorders, kidney dysfunctions, viral hepatitis, cirrhosis, autoimmune hepatitis, Wilson's disease, drug-induced or any other hepatic diseases, hemochromatosis, malignant tumors, taking antioxidant supplements (except for vitamin E) like vitamin C, selenium, carotenoids, as well as omega-3 supplement for a minimum of six months before participation in the study or throughout the trial, receiving lipid-lowering and antihypertensive medications; being on a calorie-restricted diet, smoking or being exposed to cigarette smoking, and unwillingness to continue the study.

Prior to the commencement of the study, the objectives and protocol of the study were fully clarified for the participants, and the subjects were asked to sign a written informed consent. The study protocol was approved by the ethics committee of Tabriz University of Medical Sciences (reference number: TBZMED.REC.1394.786) and registered in the website of Iranian registry of clinical trials (available at http://www.irct.ir, identification ID: IRCT201511143320N12).

2.2. Sample Size Calculation

The sample size was calculated according to the changes in triglyceride (TG) levels obtained from the study by Zhang et al. (8). Considering a power of 80% and confidence level of 95%, a sample size of 21 patients was calculated in each group by formula: n = ((Z1-α/2 + Z1-β)2 × (SD12+ SD22)) / d2. Taking into account a probable dropout of 20%, the sample size was increased to 25 subjects in each group.

2.3. Study Design

This study was designed as a double-blind, placebo-controlled randomized clinical trial with a 1:1 allocation ratio. The participants, who met the inclusion criteria, were

randomly assigned to intervention or placebo groups by random allocation software (RAS), using a random block procedure, which consisted of four subjects per block and matched subjects to each block considering their age, gender, and BMI. A statistician, who had no role in the clinical procedures of the study conducted the allocation. Once the random sequences were generated by the software, they were kept in a secure location and managed by a third party, who had no involvement in the study. Then, the codes were printed on the supplement boxes, and the coded bottles were delivered to the eligible patients. The patients in the intervention group received 1200 mg/day ALA supplement divided to two capsules (one, 30 minutes before breakfast and one, 30 minutes before dinner) plus 400 mg of vitamin E. Participants in the placebo group received placebo capsules (containing starch) at the same regimen, plus 400 mg vitamin E; the study duration was 12 weeks. Study capsules (42 capsules in each opaque plastic bottle) were given every three weeks (provided at the beginning, 3rd, 6th and 9th weeks) and instructions were given on storage and consumption of the capsules. The capsules and the containers for ALA capsules and the placebos were the same regarding shape, color, and volume. The subjects were followed on a weekly basis by telephone to the confirmed regular taking of the capsules and enquired about any side effects during the study. To avoid any bias, the patients, radiologist, researchers and those involved in enrolling, filling out the questionnaires, treating and assessing the participants were all blinded to the capsules each patient received. The participants' compliance with the capsules was assessed by requesting the subjects to bring with them the medication containers at each visit; the remaining capsules were counted and a loss of more than 10% of the capsules was regarded as incompliance, and the patient was no longer included in the study.

2.4. ALA and Placebo Capsules Preparation

Hard yellow gelatin capsules were used as a delivery vehicle in the present study. The ALA supplements contained > 98% R, S- ALA and were in the form of 600-mg capsules. The placebo capsules, which were identical to the ALA supplements in color and size were supplied by the school of pharmacy, Tabriz University of Medical Science, Tabriz, Iran.

2.5. Demographic Characteristics and Anthropometric Measurements

At baseline, demographic characteristics, including age, gender, marital and educational status were obtained for each subject. Body weight and height were measured with minimal clothes and no shoes on using the Seca scale (Hamburg, Germany) with a precision of 100 g and 0.5 cm, respectively; body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (WC) measurement was performed at the midpoint between the iliac crest and the lowest rib in standing position, using an unstretched tape measure with a 1-mm precision. All the anthropometric measurements were performed at baseline and end of the study by the same assessor. All the measuring equipment were calibrated prior to the beginning of the study.

2.6. Dietary Intake and Physical Activity Assessment

Dietary intake data were collected using a three-day 24-hour dietary recall (two working days and a weekend) at baseline and end of the study. Food intake was analyzed by a Nutritionist IV software modified for Iranian foods (First Databank; Hearst, San Bruno, CA, USA). Physical activity levels were estimated based on the data obtained from the short form of the international physical activity questionnaire (IPAQ), at baseline and after 12 weeks of intervention, and then categorized as "high," "moderate," and "low" activity (14).

2.7. Biochemical Measurements

At baseline and end of the study, ten mL of blood was taken from the antecubital vein after a 12-hour overnight fast, and serum samples were separated by centrifugation at 3000 rpm for 15 minutes (Hettich D-78532, Tuttlingen, Germany) at room temperature. The FSG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) concentrations were analyzed on the same day of sampling, and the remaining sera samples were stored at -80°C until the assays. The FSG, TC, HDL-C, TG, ALT, AST, and ALP concentrations were measured via the enzymatic colorimetric method using an automatic analyzer (Abbott, model Alcyon 300, USA) by commercial kits (Pars-Azmoon Co., Tehran, Iran). Serum concentrations of low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald formula (15). Substitute measures assessed Beta-cell function and insulin sensitivity, Homeostasis Model Assessment (HOMA) of β-cell function (HOMA-B) index and quantitative insulin sensitivity check index (QUICKI), respectively. The HOMA-B and QUICKI were estimated by the suggested formulas (16, 17).

2.8. Statistical Analysis

The results were expressed as the mean $\pm$ standard deviation (SD) for quantitative data with normal distribution, and frequency (percent) for qualitative data. The normal distribution of the variables was checked using

the Kolmogorov-Smirnov test. Based on the result of this test, all variables in this study were normally distributed. Results of the changes in quantitative variables within and between groups, throughout the study are presented as mean differences (MDs) with 95% confidence intervals (CIs). The analyses were performed by intention-to-treat approach (ITT), and there were no missing data on the final analysis. To compare differences between the study groups at baseline, chi-square test and independent sample's t-test were used for categorical and continuous data, respectively. The Fisher's exact test was used instead of the chi-square test to compare the two groups for qualitative variables that did not follow the Cochrane criteria.

The within-group changes were analyzed using the paired sample t-test for quantitative variables. To omit the effects of confounders, the researchers used analysis of covariance (ANCOVA) to determine the effects of ALA supplementation by adjusting for the confounders (Baseline values and BMI, energy intake, and physical activity changes throughout the study). All statistical analyses were performed using the SPSS software (IBM Statistics for Windows, version 21.0, IBM Corp., Armork, N.Y., USA), and statistical significance was set at P value < 0.05.

3. Results

3.1. Patient Characteristics

From 50 patients included in the randomization process (25 in the ALA group and 25 in the placebo group), two subject in the ALA group and three subjects in the placebo group discontinued the study because of cessation of intervention, and finally, 23 subjects in the ALA group and 22 patients in the placebo group completed the trial (Figure 1). Capsule counts indicated good compliance on the part of the patients, who completed the study, and about 98% and 96% of capsules in the ALA group and placebo group were consumed, respectively. Patients did not report any unfavorable effects or symptoms following ALA or placebo consumption throughout the study.

The baseline characteristics of the study patients are shown in Table 1. The mean age in the ALA group and placebo groups were 40.6 ± 5.6 years and 38.8 ± 6.5 years, respectively, and no difference in gender ratio was found between the groups at baseline.

The mean energy and carbohydrate intake significantly decreased in both groups while protein intake decreased only in the placebo group ($P < 0.05$ for all). No statistically significant differences were found for energy and macronutrient intakes between the groups neither at baseline nor the end of the study (energy: -192 vs. -195 kcal/day; carbohydrate: -26.4 vs. -27.8 g/day; protein: 11.3 vs. 13.6 g/day; total fat: 3.5 vs. 5.5 g/day) ($P > 0.05$) (data not shown). Furthermore, physical activity level was not significantly different within and between the two groups throughout the study period ($P > 0.05$) (data not shown).

3.2. Anthropometric Measurements

The results indicated a significant reduction in weight, BMI, and WC in both groups ($P < 0.0001$ for all); however, no significant differences were observed between the groups after adjusting for the confounders (data not shown).

3.3. Glycemic Markers

Compared with baseline, FSG ($P = 0.004$), insulin ($P = 0.010$) and insulin sensitivity (QUICKI) ($P = 0.007$) improved after the intervention in the ALA group, whereas the glycemic markers were not altered significantly within the placebo group (Table 2). Furthermore, the HOMA-B scores did not change substantially in either of the groups. The results of analysis of covariance (ANCOVA) test after adjusting for the baseline values as well as other confounders showed that serum insulin was significantly decreased ($P = 0.019$), and QUICKI scores were significantly increased ($P = 0.033$) in the ALA group compared to the placebo group; no significant differences were revealed for FSG (Table 2).

3.4. Lipid Profile

Serum TG, TC, LDL-C, and LDL/HDL ratio were significantly reduced in the ALA group ($P < 0.05$ for all), while no significant changes were found for these factors in the placebo group (Table 2). Only serum levels of TG were significantly different between the groups after adjustment for confounders ($P = 0.037$) (Table 2).

3.5. Liver Enzymes

Table 3 shows that serum ALT, AST, and ALP significantly decreased in the ALA group ($P < 0.05$ for all), whereas only a reduction in serum ALT ($P = 0.034$), but not AST and ALP, was observed in the placebo group. Regarding AST/ALT ratio, no significant changes were observed within neither of the groups. Adjustment for the confounders revealed that changes in liver enzymes and AST/ALT ratios were not significantly different between the two groups (Table 3).

4. Discussion

The present study assessed the potential effects of daily supplementation with 1200 mg ALA for 12 weeks on NAFLD and revealed that ALA supplementation resulted in improved insulin sensitivity as well as a significant decrease

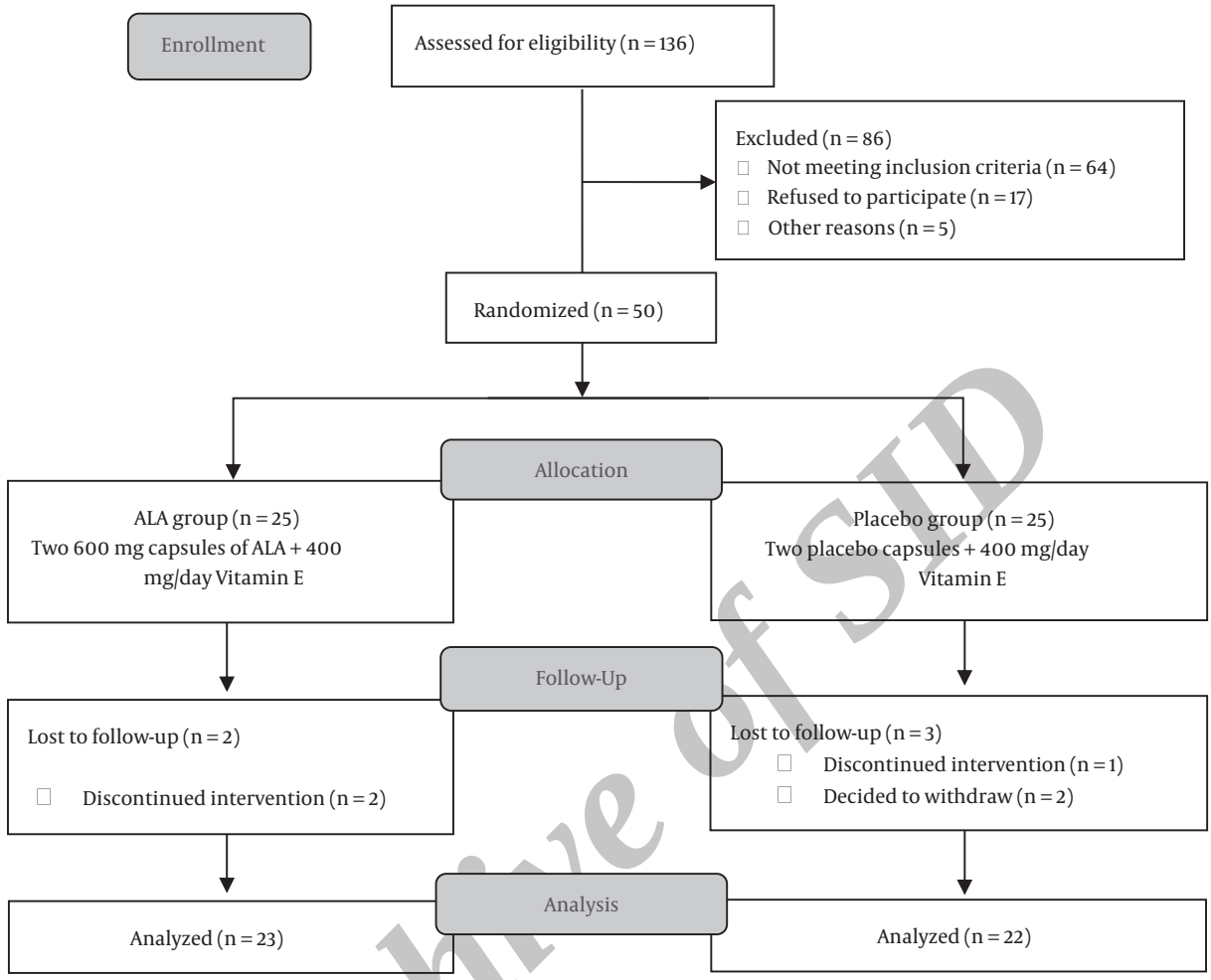

Figure 1. Flow diagram of the study

in serum insulin and TG, yet did not affect other lipid profile parameters, FSG, HOMA-B, hepatic enzymes, and anthropometric measurements in patients with NAFLD.

Although the beneficial effects of ALA have been shown in vitro and animal studies (10, 18-20), clinical trials appear to be rare in this field. Furthermore, IR is now considered an essential condition in the pathophysiology of NAFLD and leads to impaired lipid and glucose metabolism (5). Hyperinsulinemia, caused by elevated insulin secretion and diminished insulin degradation by the liver, is probably a compensatory event to IR, and it is associated with increased TG uptake by the liver and decreased fatty acid oxidation in this organ. This condition can result in adverse metabolic outcomes, such as further reduction in insulin signaling and eventually impaired glucose and lipid metabolism (21). The current results indicated that ALA supplementation for 12 weeks in obese patients with NAFLD led to considerable decreases in serum insulin levels accompanied by a significant increase in QUICKI scores in comparison with the placebo, while no effect on FSG was observed. Previous studies have reported on the beneficial effects of ALA administration on glucose metabolism and insulin sensitivity, especially in diabetic patients. Rett et al. (22) investigated acute intravenous infusion with ALA 500 mg in Type 2 Diabetes Mellitus (T2DM) patients and reported enhanced insulin sensitivity. Furthermore, daily administration of 600 mg ALA, twice daily for four weeks, improved glucose levels and insulin sensitivity in obese and lean T2DM patients (23). Ansar et al. (24) examined the effects of ALA supplementation (300 mg daily) for eight weeks in patients with T2DM and reported increased insulin sensitivity. The current results, similar to numerous

Table 1. General Characteristics of Participants at Baseline[a,b]

Characteristics	ALA group (N = 23)	Placebo group (N = 22)	P Value
Age, y	40.6 ± 5.6	38.8 ± 6.5	0.342[c]
Male, N. (%)	11 (47.8)	12 (54.5)	0.652[d]
Marital Status, N. (%)			0.096[e]
Single	1 (4.3)	5 (22.7)	
Married	22 (95.7)	17 (77.3)	
Highest Education completed, N. (%)			0.279[d]
Primary	5 (21.7)	8 (36.4)	
Diploma and higher	18 (78.3)	14 (63.6)	
Physical activity			0.378[d]
Low	3 (13.0)	2 (9.1)	
Moderate	18 (78.3)	17 (77.3)	
High	2 (8.7)	3 (13.6)	
Weight, kg	92.2 ± 11.0	93.7 ± 15.4	0.706[c]
Height, cm	165.2 ± 8.4	165.5 ± 9.4	0.905[c]
Body mass index, kg/m^2	33.8 ± 3.7	34.1 ± 4.5	0.797[c]
Waist circumference, cm	107.5 ± 7.1	106.7 ± 8.2	0.708[c]
Energy, kcal/day	2249 ± 530	2385 ± 447	0.360[c]
Carbohydrate, g/day	344.9 ± 83.4	353.9 ± 73.7	0.704[c]
Protein, g/day	72.4 ± 23.5	78.7 ± 18.6	0.321[c]
Fat, g/day	57.0 ± 19.9	64.9 ± 11.2	0.110[c]
Liver steatosis grade, N. (%)			0.224[d]
Grade 1	5 (21.7)	8 (36.4)	
Grade 2	10 (43.5)	11 (50)	
Grade 3	8 (34.8)	3 (13.6)	

Abbreviation: ALA, Alpha-Lipoic Acid.
[a]Values are expressed as mean ± SD unless indicated.
[b]P < 0.05 was considered significant.
[c]P values obtained from Independent sample t-test.
[d]P values obtained from Pearson's chi-squared test.
[e]P values obtained from Fisher's exact test.

in vitro (25, 26) and animal studies (27-29), indicated that ALA improves insulin sensitivity. However, in a randomized clinical trial, de Oliveira et al. (24) failed to show any significant changes in HOMA index following oral supplementation with 600 mg ALA, once daily in T2DM patients.

The mechanism by which ALA improves insulin sensitivity in NAFLD patients could be partly due to its antioxidant properties (30). Studies have demonstrated that oxidative stress plays a pivotal part in insulin resistance through inhibition of insulin-induced insulin receptor tyrosine phosphorylation and insulin receptor substrate one phosphorylation (30). Furthermore, ALA, by stimulating glucose uptake in both skeletal muscle (31) and adipose tissue (32), enhances translocation of the GLUT1 and GLUT4 in 3T3-L1 adipocytes and L6 myotubes (33) and modulates insulin signaling pathways by stimulating AMPK, PI3-kinase, and protein kinase B (Akt) activities in the skeletal muscle (34) and adipose tissue (32).

In the current study, after 12 weeks of supplementation with ALA, serum insulin levels significantly diminished in the ALA group in comparison with the placebo group, yet FSG did not differ between the groups. This was in accordance with Song et al.'s (27) study, in which ALA was administrated to obese Otsuka Long-Evans Tokushima fatty (OLETF) rats for three weeks, and a significant decrease was reported in plasma insulin. In the animal model of Jung et al.'s study (20), hyperinsulinemia in OLETF rats was found to be significantly reversed by ALA administration. How-

Table 2. The Effect of ALA on Glycemic Markers and Lipid Profile on Obese Patients with NAFLD Throughout the Study[a,b]

Variables	ALA Group (N = 23)	Placebo Group (N = 22)	MD (CI 95%)	P Value
FSG, mg/dL				
Baseline	100.0 ± 13.9	93.5 ± 10.4	6.4 (-1.0, 13.9)	0.086[d]
After 12 weeks	93.6 ± 12.9	90.3 ± 10.3	-1.1 (-6.4, 4.3)	0.690[e]
MD (CI 95%)	-6.4 (-10.5, -2.3)	-3.3 (-7.1, 0.6)		
P value[c]	0.004	0.094		
Insulin, μIU/mL				
Baseline	18.2 ± 9.7	19.3 ± 9.5	-1.1 (-6.9, 4.6)	0.691[d]
After 12 weeks	13.4 ± 5.4	18.1 ± 8.6	-4.2 (-7.6, -0.7)	0.019[e]
MD (CI 95%)	-4.7 (-8.2, -1.2)	-1.2 (-4.3, 2.0)		
P value[c]	0.010	0.445		
QUICKI				
Baseline	0.314 ± 0.022	0.313 ± 0.022	0.001 (-0.012, 0.014)	0.917[d]
After 12 weeks	0.329 ± 0.025	0.317 ± 0.020	0.012 (0.001, 0.023)	0.033[e]
MD (CI 95%)	0.01 (0.004, 0.025)	0.003 (-0.003, 0.010)		
P value[c]	0.007	0.290		
HOMA-B				
Baseline	203.8 ± 140.1	257.3 ± 171.5	-53.5 (-147.5, 40.4)	0.257[d]
After 12 weeks	187.5 ± 125.6	254.9 ± 124.0	-42.1 (-107.5, 23.4)	0.202[e]
MD (CI 95%)	-16.3 (-58.0, 25.3)	-2.4 (-74.9, 70.1)		
P value[c]	0.426	0.946		
Triglycerides, mg/dL				
Baseline	181.0 ± 84.4	198.6 ± 73.4	-17.6 (-65.2, 30.1)	0.461[d]
After 12 weeks	146.9 ± 60.6	186.3 ± 54.2	-31.1 (-60.4, -1.9)	0.037[e]
MD (CI 95%)	-34.1 (-62.1, -6.0)	-12.3 (-45.4, 20.9)		
P value[c]	0.019	0.450		
Cholesterol, mg/dL				
Baseline	189.3 ± 29.2	189.4 ± 42.6	-0.1 (-21.9, 21.8)	0.996[d]
After 12 weeks	174.5 ± 22.8	181.2 ± 31.5	-6.6 (-20.9, 7.8)	0.361[e]
MD (CI 95%)	-14.8 (-25.9, -3.7)	-8.1 (-24.3, 8.0)		
P value[c]	0.011	0.306		
LDL-C, mg/dL				
Baseline	109.7 ± 27.2	101.2 ± 40.3	8.5 (-12.1, 29.1)	0.408[d]
After 12 weeks	95.9 ± 24.4	93.9 ± 31.6	-2.2 (-16.8, 12.3)	0.759[e]
MD (CI 95%)	-13.8 (-25.4, -2.3)	-7.3 (-21.6, 6.9)		
P value[c]	0.021	0.298		
HDL-C, mg/dL				
Baseline	46.4± 9.1	48.4 ± 8.7	-2.0 (-7.4, 3.3)	0.452[d]
After 12 weeks	48.8 ± 8.7	50.1 ± 9.9	0.1 (-4.3, 4.5)	0.965[e]
MD (CI 95%)	2.4 (-0.7, 5.6)	1.6 (-1.8, 5.1)		
P value[c]	0.120	0.332		
LDL/ HDL ratio				
Baseline	2.4 ± 0.9	2.1 ± 0.9	0.3 (-0.3, 0.8)	0.330[d]
After 12 weeks	2.0 ± 0.6	1.9 ± 0.7	-0.033 (-0.37, 0.31)	0.846[e]
MD (CI 95%)	-0.37 (-0.72, -0.02)	-0.19 (-0.49, 0.11)		
P value[c]	0.038	0.203		

Abbreviations: ALA, Alpha-Lipoic Acid; CI, Confidence Interval; FSG, Fasting Serum Glucose; HDL-C, High Density Lipoprotein; HOMA-B, Homeostasis Model Assessment of β-Cell Function; LDL-C, Low Density Lipoprotein; MD, Means Differences; QUICKI, Quantitative Insulin Sensitivity Check Index.
[a]Values are expressed as mean ± SD.
[b]P < 0.05 was considered significant.
[c]P values indicate comparison within groups (Paired t-test).
[d]P values indicate comparison between groups at baseline (Independent sample t-test)
[e]P values indicate comparison between groups after intervention (ANCOVA; adjusted for baseline values, energy intake and BMI changes).

Table 3. The Effect of ALA on Liver Enzymes Measures of Obese Patients with NAFLD Throughout the Study[a,b]

Variables	ALA Group (N = 23)	Placebo Group (N = 22)	MD (CI 95%)	P Value
ALT, IU/L				
Baseline	32.8 ± 16.2	32.5 ± 18.9	0.3 (-10.3, 10.8)	0.958[d]
After 12 weeks	26.5 ± 14.3	25.9 ± 11.2	0.7 (-0.5, 6.5)	0.799
MD (CI 95%)	-6.3 (-11.4, -1.2)	-6.7 (-12.8, -0.5)		
P value[c]	0.017	0.034		
AST, IU/L				
Baseline	23.7 ± 8.9	23.8 ± 9.8	-0.08 (-5.7, 5.5)	0.977[d]
After 12 weeks	19.6 ± 7.6	20.6 ± 4.8	-0.9 (-4.1, 2.3)	0.592[e]
MD (CI 95%)	-4.1 (-6.9, -1.3)	-3.2 (-7.0, 0.5)		
P value[c]	0.006	0.089		
ALP, IU/L				
Baseline	211.3 ± 44.7	207.5 ± 49.7	3.8 (-24.6, 32.2)	0.791[d]
After 12 weeks	199.1 ± 54.1	196.9 ± 37.1	-1.2 (-15.7, 13.3)	0.869[e]
MD (CI 95%)	-12.2 (-22.7, -1.7)	-10.6 (-21.3, 0.1)		
P value[c]	0.025	0.053		
AST/ ALT ratio				
Baseline	0.803 ± 0.26	0.869 ± 0.38	-0.066 (-0.26, 0.13)	0.496[d]
After 12 weeks	0.801 ± 0.22	0.911 ± 0.34	-0.074 (-0.21, 0.06)	0.262[e]
MD (CI 95%)	-0.002 (-0.08, 0.08)	0.042 (-0.09, 0.17)		
P value[c]	0.957	0.516		

Abbreviations ALA, Alpha-Lipoic Acid; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; CI, Confidence Interval; HC, Hip Circumference; MD, Means Differences; WC, Waist Circumference; WHR, Waist-to-Hip Ratio.
[a] Values are expressed as means ± SD.
[b] $P < 0.05$ was considered significant.
[c] P values indicate comparison within groups (Paired t-test).
[d] P values indicate comparison between groups at baseline (Independent sample t-test)
[e] P values indicate comparison between groups after intervention (ANCOVA; adjusted for baseline values, energy intake and BMI changes).

ever, there are studies, which failed to show the effect of ALA supplementation on serum insulin levels (24, 35) that might be attributed to varying methodologies of the studies and different physiological mechanisms between animals and human in insulin metabolism. Although the exact mechanism is unknown, improved insulin sensitivity is likely to mediate ALA's potential benefit on serum insulin levels.

In contrast to the current results, Ansar et al. (24) reported a significant decrease in fasting blood glucose of patients with T2DM, following ALA supplementation. In addition, a 16-week supplementation with 600 mg/day ALA in T2DM patients caused a significant reduction in serum glucose levels (35). However, no significant changes in fasting plasma glucose levels were reported by Jacob et al. (36) after administering intravenous ALA daily for ten days in patients with T2DM; this finding was similar to the study on patients with NAFLD.

Dyslipidemia along with other characteristics of metabolic syndromes, such as obesity and impaired glucose tolerance, is one of the most prevalent metabolic disorders in patients with NAFLD. Numerous animal studies have reported that ALA has blood lipid modulating properties beyond its antioxidant and anti-inflammatory features (37, 38). In the current study, only a significant decline in serum TG was observed in the ALA group in comparison with the placebo group. In a previous short-term clinical trial, supplementation with ALA (400 mg/day for four weeks) had no influence on serum TC and TG in patients with T2DM; yet HDL-C concentration was significantly affected (39). Also, oral supplementation with 600 mg ALA for eight weeks in hemodialysis patients enhanced serum HDL-C levels, yet did not affect other lipid profile components (40). Moreover, Zhang et al. (8) reported decreased serum TC, TG, and LDL-C levels in obese subjects, following ALA supplementation.

The underlying mechanism by which ALA could improve lipid profile is not completely elucidated. The probable mechanism is increased insulin sensitivity and controlled activities of the enzymes involved in lipolysis and triglyceride synthesis (41). Furthermore, ALA appears to increase activities of lipoprotein lipase as well as Lecithin Cholesterol Acyltransferase (LCAT) (42).

In this study, the researchers assessed liver enzymes as

well as AST/ALT-ratio as biochemical markers of liver function. There is limited evidence of the effect of ALA supplementation on these biomarkers. However, Sivaprasad et al. (43), similar to the current study, reported that ALA could not change liver enzymes in rats with lead-induced lipid peroxidation. In contrast to the current findings, adding ALA to the diet of rats receiving a high-fat diet resulted in the noticeable reduction in ALT and AST (44). To the best of the author's knowledge, this study appears to be the first study that examined the effect of ALA supplementation on liver function; therefore, the data could not be compared with other human studies.

4.1. Strengths and Limitations

The present study was the first report about the efficacy of ALA supplementation on liver function and metabolic markers in obese patients with NAFLD. In addition, relatively high compliance rate, adjustment for the confounders, sufficient study duration and methodology as double-blind, and placebo-controlled design are considered as valuable strengths of the study. Nevertheless, the current study had some limitations, including relatively small sample size, lack of measurement of serum ALA and the use of ultrasonography to diagnose NAFLD instead of tissue biopsy.

4.2. Conclusion

The present study could show the beneficial effects of ALA supplementation on improving lipid profile and insulin sensitivity in patients with NAFLD. Therefore, ALA might decrease the risk of developing CVD in NAFLD patients by modulating lipid profile and improving insulin resistance.

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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 preparation of the manuscript, and did the statistical analyses. Sonya Hosseinpour-Arjmand assisted the measurement of biochemical values, gathering of data, especially anthropometric and dietary intake data, and coordinated in the delivery of interventions. Mehrangiz Ebrahimi-Mameghani provided assistance in the design of the study, supervised the data collection, and edited the manuscript. All authors participated in data interpretation and read and approved the content of the manuscript.

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