Effect of alpha-lipoic acid on blood glucose, insulin resistance, and glutathione peroxidase of type 2 diabetic patients

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ABSTRACT

الأهداف: تقييم تأثير استخدام حمض الألفا لينولينك لمدة شهرين على غلوكوز الدم، ومقاومة الأنسولين، ونشاط آز بروكسيد الغلوتوثيون وذلك بغرض علاج المرضى المصابين بالسكري من النمط الثاني.

الطريقة: أجريت هذه الدراسة في عيادة متاهاري التابعة لجامعة شيراز للعلوم الطبية، شيراز، إيران، واستمرت خلال الفترة من مايو إلى أكتوبر 2006م. لقد تم تقسيم المشاركين (العدد=57) في الدراسة بطريقة العينة العشوائية المنتظمة إلى المجموعتين: مجموعة المرضى الذين تم علاجهم بحمض الألفا لينولينك لمدة 8 أسابيع (300 ملغ يومياً)، ومجموعة المرضى الذين تم علاجهم بالغفل ولمدة 8 أسابيع أيضاً. لقد تم سحب عينات الدم من أجل تحليل نتائج كلاً من: تحليل سكر الدم بعد ليلة كاملة من الصيام وبعد ساعتين من الأكل، واختبار مستويات الأنسولين في مصل الدم، ونشاط آز بروكسيد الغلوتوثيون.

النتائج: أشارت نتائج الدراسة إلى الانخفاض الواضح في مستويات الغلوكوز بعد ليلة كاملة من الصيام وبعد ساعتين من الأكل، بالإضافة إلى انخفاض مقاومة الأنسولين ومستويات آز بروكسيد الغلوتوثيون في المجموعة التي تم علاجها بحمض الألفا لينولينك. كما كان هناك انخفاضاً ملحوظاً في مستويات الغلوكوز بعد الصيام، ومقاومة الأنسولين في المجموعة التي تم علاجها بحمض الألفا لينولينك عند مقارنتها مع مجموعة الغفل.

خاتمة: أثبتت هذه الدراسة مدة فعالية استخدام حمض الألفا لينولينك كمضاد للأكسدة من أجل علاج المرضى المصابين بالسكري.

Objectives: To examine the effects of alpha-lipoic acid (ALA) treatment over a period of 2 months on fasting blood glucose (FBG), insulin resistance (IR), and glutathione peroxidase (GH-Px) activity in type 2 diabetes (T2DM) patients.

Methods: This study took place in Motahari Clinic, Shiraz, Iran, which is affiliated to Shiraz University of Medical Sciences from May to October 2006. Type 2 DM patients (n=57) were divided into 2 groups to receive either ALA (300 mg daily) or placebo by systematic randomization, and were followed-up for 8 weeks. After an overnight fasting and 2 hours after breakfast, patients' blood samples were drawn and tested for FBG, 2 hours post-prandial plasma glucose, serum insulin level, and GH-Px activity.

Results: The result of the study showed a significant decrease in FBG and PPG levels, IR-Homeostasis Model Assessment (IR-HOMA index), and GH-Px level in the ALA group. The comparison of differences between FBG and IR at the beginning and at the end of study in the ALA treated group and the placebo group were also significant.

Conclusion: This study supports the use of ALA as an antioxidant in the care of diabetic patients.

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Hyperglycemia, resulting from defective insulin secretion, insulin resistance, or both are characteristics of diabetes mellitus (DM) as a metabolic disease.[1] The incidence is increasing dramatically all over the world, and is linked to reduced quality of life and increased mortality and morbidity.[2] Studies showed that diabetes is strongly associated with increased oxidative stress, which is either the consequence of increased production of free radicals, or reduced antioxidant defense.[3] Oxidative stress is associated with a number of pathological conditions including atherosclerosis, cardiovascular, and neurodegenerative diseases. The stability and capacity of the antioxidant status in chronic diabetes influence the outcome of long term complications caused by oxidative stress.[4] Oxidative stress not only is connected to many complications induced by diabetes,[5] but also has been linked to insulin resistance (IR) through activation of stress-sensitive signaling pathways.[6] Decrease in insulin sensitivity is a core defect in type 2 DM (T2DM), leading to diminished glucose uptake and disposal in peripheral tissues, and increased hepatic glucose production in the liver.[7] The concept of oxidation stress as an important trigger in the onset and progression of diabetes, may propose a unique therapeutic option to treat the disease and its complications by using antioxidants, or nutrients with high antioxidant capacity.[8] Alpha-lipoic acid (ALA), a biological antioxidant, and natural co-factor of mitochondrial dehydrogenase complexes,[7] has been the focus of research in nutrition and diabetes in the last few years.[9] This potent antioxidant is an essential co-factor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase (PDH) complex.[10] The ALA is absorbed from the gastrointestinal tract easily, and it is able to cross the blood brain barrier without any serious side effects.[11] It has been shown to improve glucose metabolism in diabetic patients.[12,13] Additionally, in experimental animals, ALA restored insulin stimulated glucose uptake into IR skeletal muscles, and as a result may act as a hypoglycemic agent through basal and insulin-activated glucose uptake.[14,15]

The present study was designed to investigate the effect of ALA supplementation on plasma glucose, IR, and glutathione peroxidase (GH-Px) in T2DM patients.

Methods. The study population composed of 14 men and 43 women, with a mean age of 53 years, and who had been diagnosed with T2DM by an endocrinologist. This study took place in Motahari Clinic, Shiraz, Iran, which is affiliated to Shiraz University of Medical Sciences from May to October 2006. Diabetes was defined according to the recommendations of the American Diabetes Association's Expert Committee on the Classification and Diagnosis of Diabetes.[1] Patients were included in the study according to the following inclusion criteria: diabetes' duration >1 year; and a fasting blood glucose (FBG) >126 mg per deciliter. Patient's who had prior history of coronary artery disease, significant vascular heart disease, congestive heart failure, severe hepatic or renal disease, malignancy, nutritional deficiency, mood disorder, and history of smoking in the last 6 months were excluded. All patients gave their written informed consent to participate, and the protocol was approved by the Shiraz University of Medical Sciences research committee. All patients were treated with stable dose of anti-diabetic medication, and some of them took antihypertensive drugs (calcium channel blockers). This systematic randomized, double-blind, placebo-controlled trial took a total duration of 2 months.

Treatment with ALA. Patients were randomized systematically to 300 mg ALA (6 men, 23 women), or a corresponding placebo (8 men and 20 women) content of 300 mg of calcium phosphate. Capsules were taken daily before food intake (both ALA and placebo capsules looked identical). Three patients were excluded from the study as they did not return for the final visit.

Biochemical measurement. Fasting and 2-hour post-prandial plasma glucose (PPG) (2 hours after breakfast) concentrations were measured with a glucometer (Auto Analyzer RA; Cobas 1000, Roche, London, UK) at the beginning of the study, and at the end of 8 weeks. Insulin levels were determined using enzyme linked immunosorbent assay (ELIZA) method kit (DRG Instruments GmbH, Frauenbergstr, Marburg, Germany). The IR was determined by the Homeostasis Model Assessment (HOMA) as an index of IR, calculated by the following formula: (Insulin [in μU/mL] X glucose [in mmol/L])/22.5).[16] Erythrocyte GH-Px activity was determined spectrophotometrically (UV method) (Randox Laboratories UK Ltd, Crumlin, Co Antrim, UK), and it was expressed as GH-Px utilized/mg/Hb/min.

Statistical analysis was performed using Statistical Package for the Social Sciences version 14 (SPSS Inc. Chicago, IL, USA). For descriptive purposes, mean ± standard deviation (SD) values are given. Data were analyzed by paired-sample t-test for changes of variables in the patients before and after ALA treatment. The independent-sample t-test was used to compare and determine statistically significant differences between the T2DM patients in both cases and controls. The power of the study was 95%. $P<0.05$ was considered as significant level of difference.

Results. The baseline demographics data and gender frequency distribution of the subjects in both experimental and placebo groups are shown in **Table**

1. All T2DM patients had no significant difference in age and duration of disease (**Table 1**). There was also no substantive differences between the 2 groups for the body weight and body mass index. Hypoglycemic drugs, nutrition, and type and level of exercise did not change during the study. **Table 2** shows changes in biochemical markers after ALA treatment. The FBG (p=0.0001) and post-prandial plasma glucose (PPG) level (p=0.023) decreased significantly after 8 weeks of ALA treatment. Fasting plasma insulin level had no change. There was also a statistically significant reduction in insulin resistance (p=0.043) and GH-Px level (p=0.035) in the experimental group. The 8 weeks treatment with ALA changed the body weight significantly (p=0.0001). This noticeable decrease in body weight was seen in controls, as well (p=0.0001) (**Table 3**). **Table 3** shows the change of variables in controls. It can be seen that there was no significant decrease in parameters with the exception of weight. Comparison of the differences between weight, fasting, PPG, IR, and GH-Px level at baseline, and at the end of trial in the experimental and placebo groups have been demonstrated in **Table 4**. The differences in fasting plasma glucose (p=0.001), and IR (p=0.006) were significant. There was also no considerable difference in weight, PPG, IR, and GH-Px activity level between the ALA T2DM patients and the placebo group.

Table 1 - Clinical characteristics of patients in the ALA and placebo groups.

Variables	ALA n=29	Placebo n=28	*P*-value†
Gender, n (%)			
Men	6 (20.7)	8 (28.6)	
Women	23 (79.3)	20 (71.4)	
Age, years*	49 ± 9.07	51.82 ± 8.25	0.254
Duration of disease, months*	96.28 ± 70.8	72.43 ± 61.64	0.181
Weight, kg*	69.9 ± 11.67	72.6 ± 13.23	0.41
BMI, kg/m²*	27.6 ± 4.2	28.02 ± 5.37	0.781

*mean ± standard deviation, †difference between the 2 groups, ALA - alpha-lipoic acid, BMI - body mass index

Table 2 - Change in variables at baseline and after the intervention period in ALA-treated patients (n=29).

Variables	Baseline	After 8 weeks	*P*-value*
Weight, kg	69.90 ± 11.67	67.82 ± 11.30	0.0001†
FBS, mg/dl	185.4 ± 55.3	156.3 ± 42.5	0.0001†
PPG, mg/dl	278.8 ± 92.0	238.1 ± 97.8	0.023†
FPI, µ IU	6.39 ± 4.62	6.02 ± 3.72	0.437
IR HOMA index	2.68 ± 1.75	2.19 ± 1.18	0.044†
GH-Px, UI/mg/Hb/min	152.51 ± 22.57	137.81 ± 23.93	0.035†

Data are expressed means ± standard deviation. *indicates difference between baseline and end visit. † significant p-values. ALA - Alpha lipoic acid, FBS - fasting blood glucose, PPG - post-prandial glucose, FPI - fasting plasma insulin, IR - insulin resistance, HOMA - homeostatic model assessment, GH-Px - glutathione peroxidase

Discussion. In the present study, oral administration of ALA has shown a significant decrease in FBG and IR in T2DM patients. These observations are in agreement with a previous finding, which reported that ALA as an effective agent can improve glucose homeostasis.[17] Budin et al[18] in 2009 found that the FBG in ALA supplemented rats after 8 weeks supplementation was significantly lower than the controls. These effects may be due to its antioxidant activity. In El-Hossary et al's[11] investigation, a significant decrease of serum glucose level was observed in diabetic ALA treated rats

Table 3 - Change in variables at baseline and after the intervention period in the control group (n=28).

Variables	Baseline	After 8 weeks	*P*-value*
Weight, kg	72.60 ± 13.23	71.48 ± 13.013	0.0001†
FBS, mg/dl	175.5 ± 42.92	182.43 ± 46.87	0.358
PPG, mg/dl	236.5 ± 79.99	207.46 ± 76.89	0.056
FPI, µ IU	7.05 ± 2.74	7.75 ± 4.15	0.192
IR HOMA index	2.64 ± 1.1	3.04 ± 1.87	0.141
GH-Px, UI/mg/Hb/min	162.43 ± 26.86	153.18 ± 21.05	0.163

Data are expressed means ± standard deviation. *indicates difference between baseline and end visit. †significant p-values. ALA - Alpha lipoic acid, FBS - fasting blood glucose, PPG - post-prandial glucose, FPI - fasting plasma insulin, IR - insulin resistance, HOMA - homeostatic model assessment, GH-Px - glutathione peroxidase

Table 4 - Comparison of differences between parameters at the beginning and at the end of 8 weeks in the ALA (n=29), and the placebo (n=28) groups.

Variables	Mean decrements	Standard deviation	T-value	*P*-value
Weight, kg			-0.223	0.824
ALA	2.034	1.62		
Placebo	2.142	2.03		
FBS mg/dl			3.55	0.001*
ALA	29.137	37.50		
Placebo	-6.92	39.19		
PPG mg/dl				
ALA	40.75	91.14	0.524	0.602
Placebo	29.03	76.81		
FPI, µ IU			1.529	0.132
ALA	0.365	2.49		
Placebo	-0.703	2.78		
IR-HOMA index			2.831	0.006*
ALA	0.485	1.24		
Placebo	-0.494	1.36		
GH-Px, UI/mg/Hb/min			0.576	0.567
ALA	14.7	37.22		
Placebo	9.243	33.43		

ALA - alpha lipoic acid, FBS - fasting blood glucose, PPG - post-prandial glucose, FPI - fasting plasma insulin, IR - insulin resistance, HOMA - homeostatic model assessment, GH-Px - glutathione peroxidase

compared to the untreated group. In addition, it was previously found that this antioxidant could reduce blood glucose level in experimentally induced DM.[19] Midaoui and de Champlain in 2002[20] had demonstrated that the plasma glucose was significantly diminished in D-glucose-treated animals given an ALA supplemented diet. Konrad[21] in 2005 mentioned the role of ALA in the translocation of glucose transporters (GLUT) from the cytoplasm to the cell surface, and results in peripheral glucose disposal.[21] Another study[22] showed an attenuated PPG in the experimental group, due to an increase in skeletal muscle glucose transport activity. Plasma insulin level of T2DM patients in the present study decreased, although not significantly, however, IR was significantly diminished.

Improvement of insulin sensitivity by ALA has been reported in T2DM patients in other studies as well. Bitar et al[13] in 2004 showed that chronic administration (100mg/kg) of the antioxidant ALA partially improved the diabetes-related deficit in glucose metabolism, and the activation of some steps in insulin signaling pathway in famous genetic rat model for human T2DM. In addition, their investigation illustrated the possible association between oxidative stress and pathogenesis of IR.[13] Oral administration of ALA (600 mg twice daily for 4 weeks) has been associated with an increase in peripheral insulin sensitivity in T2DM subjects.[7] In a placebo-controlled pilot trial, a 4-week treatment with ALA at various doses resulted in an increase in insulin sensitivity by 15% with 600 mg, 14% with 1200 mg, and 22% with 1800 mg.[23]

This study like other clinical studies, confirms that supplementation with ALA has an effect on IR as well. The mechanism of action may be due to the insulin signaling pathway, such as increase in PI 3-kinase and protein kinase B (Akt).[15-17] The ALA has been shown to increase intrinsic activity of GLUT similarly to insulin. Activation of GLUT may be mediated by p38 mitogen-activated protein kinase.[14] Chronic ALA treatment increases both insulin stimulated glucose oxidation and glycogen synthesis, moreover, is associated with significantly lower plasma levels of insulin and free fatty acids.[23] A 3-hour exposure of primary cultured rat hepatocytes to R-ALA at therapeutically relevant concentrations increases pyruvate oxidation of the PDH complex and decreases gluconeogenesis and free fatty acids oxidation.[24] Pre-clinical studies have demonstrated that ALA improves glucose uptake and glucose oxidation, thus leading to an increase of adenosine triphosphate (ATP) synthesis. In Muellenbach et al's study in 2008,[25] they reported that the combination of ALA and pyridoxamine had significant effects on the reduction of FPG (23%), insulin (16%), and enhanced whole-body insulin sensitivity in obese Zucker rats. However, in a recent randomized, double-blind, placebo-controlled trial on 10 diabetic T2DM patients, supplementation with ALA and vitamin E alone and in combination, improved HOMA index with no significant changes in insulin sensitivity.[26]

This study also showed significant decrease in erythrocyte's GH-Px enzyme level in ALA, although the difference between the 2 groups was not considerable. In support of this finding, significantly higher activities of antioxidant enzymes, namely, serum peroxidase, superoxide dismutase (SOD), and catalase (CAT) were found in T2DM patients, compared to controls in several studies. It is suggested that hyperglycemia induced an increase in antioxidant enzymes, and a relationship seems to exist between diabetic complications and elevated levels of these enzymes. These antioxidant enzymes may be considered as markers for vascular injury.[27] Likidlilid et al[28] in a study on glutathione (GSH) level and GH-Px activity in diabetic patients in comparison with normal healthy group have shown that the level of red cell GSH was significantly lower in patients, whereas red cell GH-Px activity was significantly higher than age-matched normal control. Therefore, according to their suggestion any reduction of oxidative stress in diabetic patients may be helpful for slowing down the progression of cardiovascular complications.[28]

In another investigation, the assessment of the enzymatic antioxidant activities of SOD and GH-Px in T2DM showed significant reduction in SOD activity, in contrast with elevated activities of GH-Px compared to non-diabetic control. The possible explanation for over-expression of GH-Px activity induced by increased oxidation consequent on diabetes is that it could be a compensatory mechanism by the body to prevent further tissue damage.[29] On the other hand, in Dincer et al's study[30] after a 2-hour incubation of red blood cells with hydrogen peroxide as an oxidant, significant decreases were observed in the GSH level as well as in the activity of GH-Px and GSH reductase with the most decrease in the poorly controlled diabetic patients.[30]

A major limitation of our study is the relatively short period of the study, as over such a time period, changes in HbA1c are not perceptible very well.

In conclusion, a decrease in oxidative stress and cardiovascular risk factor such as IR seems to be an ideal treatment strategy in T2DM patients. The results of the present study demonstrated that an 8-week oral treatment with ALA significantly decreased FBG and IR in patients with T2DM, and supported the possibility of improvement of diabetic complications by antioxidant therapy. Additionally, these findings could inform future studies to determine the other therapeutic effects of ALA treatment and to find more molecular

mechanism, by which this is achieved. In addition, the evaluation of appropriate doses of ALA to be used as an additional therapeutic antioxidant in this population requires more studies.

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