

α - Lipoic Acid Ameliorates the Oxidative Status and Serum Iron in Diabetic Patients

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ABSTRACT: Diabetes is accompanied by various degenerative manifestations such as aging, cardiovascular disease and microvascular lesions leading to diabetic complications. These events may be related to the hyperproduction of free radicals and to a dysfunction of biological antioxidant systems. Iron can convert poorly reactive free radicals, such as H₂O₂, into highly reactive ones. The present study aimed to investigate the effect of exogenous supplementation of α -lipoic acid (LA) on the glycemic status, lipid profile and the serum levels of some antioxidants (Superoxide Dismutase (SOD), Glutathione (GSH) and vitamin C), in addition to serum iron. The studied participants were categorized into 3 groups (15 each): Non diabetic healthy volunteers, type 2 diabetic patients and type 2 diabetic patients received α -lipoic acid in a daily oral dose of 600 mg for 2 months. All participants were subjected to full history taking and complete clinical examination. Fasting blood samples were taken for determination of serum glucose (in addition to 2 hours post prandial sample), insulin, lipid profile and antioxidants as well as iron and total iron binding capacity. Homeostasis Model Assessment (HOMA) and atherogenic index were calculated. The results revealed that exogenous supplementation of LA produced non significant improvement in serum level of glucose and glycated hemoglobin, in spite of the significant improvement in serum level of insulin and HOMA index, compared to diabetic group. Lipoic acid treatment reduced significantly serum levels of TG and VLDL-C, while serum levels of cholesterol, HDL-C, LDL-C and atherogenic index revealed non significant improvement, compared to diabetic untreated group. Serum levels of the studied antioxidants and nitric oxide were elevated significantly by LA, compared to diabetic group. Supplementation with lipoic acid increased the iron level in diabetic patients toward control but this elevation was significantly lower than control.

Key Words: Type II diabetes, α -Lipoic acid, Lipid profile, Serum iron.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder, of which the number of patients is rapidly increasing worldwide due to several conditions such as aging, westernization, and increasing prevalence of obesity and physical inactivity^[1]. Diabetes is caused by inherited or acquired deficiency in insulin secretion and by decreased responsiveness of some organs to the secreted insulin^[2]. Diabetes is not simply a disorder of glucose homeostasis but is also accompanied by various degenerative manifestations which are partly due to associated abnormalities of plasma lipid and lipoprotein metabolism^[3]. Lipid peroxidation, the oxidative deterioration of the polyunsaturated fatty acids (PUFA), is increased in diabetes owing to an increase in the production of hydroperoxides, short-chain aldehydes, ketones and other oxygenated compounds^[4].

Iron is a very important transition metal for the cells in the body and its abnormal homeostasis is associated with the pathogenesis of various chronic diseases, including diabetes^[5]. Several mechanisms for the onset of diabetes and the development of diabetic complications have been

proposed, one of which may be the abnormal homeostasis of transition metals such as iron^[6].

In addition to the potential effect of iron on insulin action, insulin may also affect iron metabolism. In vitro data suggest that insulin is capable of redistributing the cellular pool of transferrin receptors, increasing the proportion at the cell surface, leading to increased cellular iron uptake in adipose tissue and the liver^[7].

Alpha lipoic acid (LA) is an endogenous short chain fatty acid functions as a cofactor of oxidative decarboxylation reactions in glucose metabolism to yield energy^[8] and has the capabilities of scavenging reactive oxygen species (ROS), chelating metal ions, stimulating insulin signaling pathway and regenerating natural antioxidants, such as glutathione and vitamins C and E^[9]. Alpha lipoic acid has generated considerable clinical interest as a cellular thiol-replenishing and redox-modulating agent^[10]. It has been used for a long time in the western world to treat complications associated with diabetes^[11].

The present study was undertaken to investigate the effect of Lipoic acid treatment on the glycemic status, lipid profile

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and the serum levels of some antioxidants (Superoxide Dismutase (SOD), Glutathione (GSH) and vitamin C), in addition to serum iron in type 2 diabetic patients.

SUBJECTS AND METHODS

Patients and treatment

The current study included 45 Egyptian males: 30 diabetic patients, who were attending the outpatient clinics of National Research Center, El-dokki health insurance polyclinic, Cairo, Egypt, their ages ranged from 40 to 55 years and 15 age, sex and culture matched apparently healthy volunteers. Written consent was obtained from all participants who were asked not to alter their usual diets and physical activities throughout the study which extended for 2 months and they were fully informed about the purpose of the study. All participants were subjected to full history taking and complete clinical examination throughout the study period.

The diagnosis of type 2 DM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus^[12]. Duration of DM ranged from 3 to 7 years. ALL patients were on metformin in a dose of 2000 mg/day and Glipimide in a dose of 2-3 mg/day. Diabetic patients were not taking any drugs other than oral anti-diabetic agents. Special stress was given to vitamins, mineral supplements, thyroid hormones, estrogen, progesterone, diuretics, or antihypertensive agents. No history of any recent acute illness or clinical evidence suggestive of kidney, liver or other endocrine and chronic diseases. Diabetic patients with chronic complications (retinopathy, symptomatic neuropathy, nephropathy and vascular diseases) were also excluded.

The studied participants were categorized into 3 groups: Control group (Control): comprising 15 non diabetic apparently healthy volunteers. Diabetic group (Diabetic): comprising 15 type 2 diabetic patients. Lipoic acid treated group (Diabetic + LA): type 2 diabetic patients (n=15), but they were convinced to receive α -lipoic acid for 2 months in a daily oral dose of 600mg. All groups were age, sex and culture matched.

Blood sampling and processing

At the end of the experiment, fasting blood samples were drawn. A part of blood sample was taken on EDTA as whole blood sample and another part was taken on a plain tube without anticoagulant for separation of serum by centrifugation at 3000 rpm for 10 min.

Serum glucose levels (fasting and 2 hours postprandial glucose) were assayed by glucose oxidase method^[13]. Glycosylated hemoglobin (Hb A_{1c}) was measured by Bio-Rad D-10TM Hemoglobin Testing System according to the method of Little *et al.*,^[14]. Serum insulin level was assayed by ELISA^[15]. Homeostasis Model Assessment (HOMA) was calculated according to Matthews *et al.*,^[16]. Serum alanine aminotransferase (ALT) and aspartate

aminotransferase (AST) activities were assayed by colorimetric method^[17]. Serum urea, creatinine and uric acid were determined according to Patton and Crouch^[18], Henry^[19] and Fossati *et al.*,^[20] respectively. Total cholesterol was determined by the enzymatic method^[13], triacylglycerol was assayed by peroxidase coupled method^[21] and HDL-C by the enzymatic method after precipitation of other lipoproteins with MgCl₂ and dextran sulphate^[22]. LDL-C was assayed by the enzymatic method after precipitation of the LDL fraction by polyvinyl sulphate^[23] while VLDL-C and atherogenic index were calculated according to Freidwald formula^[24] and Bok *et al.*,^[25] respectively. Serum total superoxide dismutase activity (SOD) was determined according to the method of Maier and Chan^[26], while serum reduced glutathione was determined by enzymatic colorimetric method^[27]. Determination of the serum level of vitamin C was performed according to the method of Jagota and Dani^[28]. Serum total Nitric oxide was assayed by enzymatic method described by Zhang and Broderick^[29]. Serum level of iron was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce, Japan) by the method of Tanner and Baranov^[30]. Serum total iron binding capacity (TIBC) was measured by Dimension RxL Max (USA). Transferrin saturation% was calculated using the equation [(serum iron / serum TIBC) X 100]^[31].

Statistical analysis

All results were expressed as the mean \pm standard error (SE). Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 13.0, Chicago, IL, USA). Comparison of different variables in various groups was done using student t test and Mann Whitney test for normal and nonparametric variables respectively. For all tests a probability (p) less than 0.05 was considered significant^[32].

RESULTS

Data illustrated in table (1) showed non significant changes in the studied liver and kidney functions in diabetic untreated group, compared to control group. Serum level of ALT in Lipoic treated group was significantly elevated (P<0.004), compared to control group, also the serum level of AST in the same group showed significant elevation (P<0.0001), compared to diabetic untreated group.

Table (1): Personal characteristics, liver and kidney function tests in control and diabetic groups (Means \pm SE).

Parameters	Control	Diabetic	Diabetic+ ALA
Age (years)	46.6 \pm 7.94	47.05 \pm 7.88	47.13 \pm 7.90
BMI (Kg m ⁻²)	28.74 \pm 2.43	30.02 \pm 1.85	29.04 \pm 1.68
Duration of diabetes (years)	---	4.13 \pm 1.41	3.93 \pm 1.39
ALT (mg/dl)	20.80 \pm 2.43	24.33 \pm 1.44	28 \pm 0.45 ^a
AST (mg/dl)	25.20 \pm 1.62	23.67 \pm 0.56	27.33 \pm 0.50 ^b
Urea (mg/dl)	24.10 \pm 0.39	29.07 \pm 1.78	25.27 \pm 0.96
Creatinine (mg/dl)	0.76 \pm 0.20	0.95 \pm 0.62	0.90 \pm 0.03

^a: Significant vs. control, ^b: Significant vs. Diabetic.

Data presented in table (2), illustrated that glucose homeostatic parameters studied in both diabetic untreated and diabetic treated groups were significantly ($P < 0.0001$) elevated, compared to control group. Non significant improvement in serum level of glucose and Hb A_{1c} was observed in LA treated group, compared to diabetic group.

Table (2): Serum levels of glucose, insulin, HOMA index and blood HbA_{1c} in control and diabetic groups (Means \pm SE).

Parameters	Control	Diabetic	Diabetic+ ALA
F. glucose (mg/dl)	76.20 \pm 1.19	167 \pm 14.60 ^a	163.20 \pm 9.99 ^a
P. glucose (mg/dl)	94.88 \pm 2.17	240.27 \pm 15.50 ^a	230 \pm 13.49 ^a
Hb A _{1c} (%)	6.53 \pm 0.18	11.33 \pm 0.99 ^a	10.26 \pm 0.56 ^a
Insulin (μ U/ml)	7.03 \pm 0.24	20.16 \pm 0.44 ^a	14 \pm 0.41 ^{a,b}
HOMA index	1.33 \pm 0.05	8.40 \pm 0.80 ^a	5.69 \pm 0.40 ^{a,b}

^a: Significant vs. control, ^b: Significant vs. Diabetic.

F. glucose: Fasting glucose, P. glucose: Postprandial glucose.

However, administration of lipoic acid improved significantly the values of insulin and HOMA index ($P < 0.001$ & 0.02 , respectively) which amounted -30.54 & -32.25% , respectively, compared to diabetic untreated group. The studied lipid profile parameters (table 3) showed significant elevations in the serum levels of cholesterol, triacylglycerol, LDL-C and VLDL-C in both diabetic groups, compared to normal control group. Serum level of HDL-C showed significant reduction ($P < 0.001$) in untreated and treated diabetic groups, compared to control group. Both diabetic groups showed significant elevation in the atherogenic index, as compared to control group. Exogenous administration of LA produced non significant improvement in serum levels of cholesterol, HDL-C, LDL-C and atherogenic index, compared to diabetic untreated group. Treatment with LA reduced significantly ($P < 0.03$) serum levels of TG and VLDL-C, compared to diabetic untreated group.

Table (3): Serum lipid profile, atherogenic index and NO in the different studied groups (Means \pm SE).

Parameters	Control	Diabetic	Diabetic+ ALA
Cholesterol (mg/dl)	189.22 \pm 12.36	222.33 \pm 16.11 ^a	210.60 \pm 11.47 ^a
TG (mg/dl)	137.38 \pm 5.20	260.67 \pm 22.99 ^a	200.40 \pm 5.92 ^{a,b}
HDL-C (mg/dl)	54.60 \pm 4.25	40.80 \pm 1.11 ^a	42.20 \pm 0.93 ^a
LDL-C (mg/dl)	107.14 \pm 10.62	151.67 \pm 4.44 ^a	141.32 \pm 5.06 ^a
VLDL-C (mg/dl)	27.48 \pm 1.04	52.13 \pm 4.60 ^a	40.08 \pm 1.18 ^{a,b}
Atherogenic Index	2.61 \pm 0.27	4.52 \pm 0.44 ^a	3.98 \pm 0.24 ^a

^a: Significant vs. control, ^b: Significant vs. Diabetic.

Atherogenic index = (Total cholesterol - HDL-C) / HDL-C

The levels of serum antioxidants are shown in Table (4). Significant reductions were noticed in the serum levels of all the antioxidant parameters (SOD, GSH and vitamin C, $P < 0.0001$) in diabetic group, compared to control group. Lipoic acid group showed non significant changes in the

serum levels of SOD and GSH, while vitamin C level showed significant reduction ($P < 0.02$), compared to control group. Exogenous supplementation of Lipoic acid improved significantly the serum levels of studied parameters, ($P < 0.001$, except for vitamin C, $P < 0.02$) compared to diabetic group. Serum level of uric acid revealed non significant reduction in diabetic group which returned to the control level by lipoic acid treatment.

Table (4): Serum levels of some antioxidants and NO in the different studied groups (Means \pm SE).

Parameters	Control	Diabetic	Diabetic+ ALA
SOD (μ g/ml)	13.09 \pm 0.83	5.68 \pm 0.19 ^a	13.48 \pm 0.70 ^b
GSH (pmol)	27.93 \pm 1.20	18.93 \pm 0.77 ^a	26.20 \pm 1.14 ^b
Vit. C (μ g/ml)	4.90 \pm 0.46	2.50 \pm 0.17 ^a	3.54 \pm 0.29 ^{a,b}
Uric acid (mg/dl)	3.69 \pm 0.30	2.78 \pm 0.12	3.14 \pm 0.16
NO (nmol/ml)	19.82 \pm 1.44	12.09 \pm 0.45 ^a	18.36 \pm 1.22 ^b

^a: Significant vs. control, ^b: Significant vs. Diabetic.

In regard to NO product, a significant reduction ($P < 0.0001$) was observed in diabetic untreated group, compared to control group. Exogenous supplementation of Lipoic acid improved significantly the serum level of NO ($P < 0.001$) compared to diabetic untreated group.

Table (5) illustrates that untreated diabetic group showed significant reduction in iron status ($P < 0.0001$) compared to control group. In regard to diabetic treated group, lipoic acid improved the iron status, compared to untreated group. Supplementation with lipoic acid improved the iron level in diabetic patients toward control but this elevation was significantly lowered than control ($P < 0.01$).

Table (5): Serum iron status in the different studied groups (Means \pm SE).

Parameters	Control	Diabetic	Diabetic+ ALA
Fe (μ g/dl)	32.60 \pm 0.71	19.20 \pm 1.02 ^a	27.40 \pm 1.40 ^{a,b}
TIBC (μ g/dl)	329.33 \pm 4.64	278 \pm 9.62 ^a	308.87 \pm 10.61 ^b
Transferrin saturation (%)	9.91 \pm 0.20	6.91 \pm 0.57 ^a	8.87 \pm 0.68 ^b

^a: Significant vs. control, ^b: Significant vs. Diabetic.

DISCUSSION

Prevalence of diabetes is globally increasing. Most of our food is broken into glucose which needs insulin to get into cells for growth and energy. In type 2 diabetes, cells do not respond appropriately to insulin^[5].

Evaluation of the catalytic activity of the liver cells, indicated by the activities of both ALT and AST, as well as the kidney function, indicated by serum levels of urea and creatinine, were conducted in order to determine the safety of the oral administration of LA. The present study revealed that serum level of ALT was significantly elevated in LA-treated group, compared to control group, while serum level of AST showed significant elevation as compared to diabetic untreated group. Despite, these changes were within the normal ranges. In addition, kidney function was significantly unaltered in all groups. Consequently, these

results indicate the safety of LA at the adopted dose level as reported by other investigators^[33].

The present results revealed significant elevations in serum levels of glucose, either in the fasting state or after 2 hours postprandial one and glycated hemoglobin, which indicates the metabolic control in the previous 3 months, as well as serum insulin level and HOMA index in diabetic group as compared with control. Despite the reduction of serum levels of glucose and glycated hemoglobin due to lipoic acid treatment, these reductions were not significant. On the other hand, the insulin sensitivity was improved significantly as indicated by the reduction of HOMA value by 37.5% in LA-treated group as compared to diabetic group.

These results agree with those of Jacob *et al.*,^[34] Konrad *et al.*,^[35] and Song *et al.*,^[36].

A possible explanation for the marginal efficacy of oral LA therapy as regard to glycemic control may be due to the short plasma half-life of LA (30 minutes) and the extensive presystemic elimination which is thought to be primarily hepatic^[37]. Furthermore, even after repeated oral administration of LA, it appears that accumulation in plasma is not achieved^[38]. Thus, following oral LA administration, a maximum plasma level is quickly reached, but falls just as quickly to a level insufficient to impact glucose control.

The precise cellular mechanisms responsible for the stimulatory effect of LA on insulin action remain unclear. Initial studies using insulin-sensitive cell lines indicated that LA activates critical elements of the insulin signaling pathways, including tyrosine phosphorylation of Insulin Receptor Substrate-1 (IRS-1) and enhancements of IRS-1 protein expression^[39]. In addition, LA proposed to recruit GLUT4 in skeletal muscles from its storage site^[40], so that glucose uptake may be stimulated by the local increase in transporter.

The present study revealed that type 2 diabetics were associated with higher concentrations of TC, TG, LDL-C and VLDL-C, as well as lower concentration of HDL-C. Oral administration of LA improved significantly serum levels of TG and VLDL-C, while serum levels of LDL-C and HDL-C improved toward the control values but with non significant manner.

Insulin is an important hormone in regulating lipid metabolism in a variety of animal tissues; it can both decrease lipolysis and cause an increase in triglyceride synthesis of adipose tissue^[41]. Krauss and Siri^[42] reported that the mechanisms responsible for hypertriglyceridaemia may be an increased hepatic secretion of VLDL and a delayed clearance of TG-rich lipoproteins, which might mainly be due to increased levels of substrates for TG production, free fatty acids and glucose. Triglyceride enrichment lipoproteins lead to increased production of the small dense form of LDL-C and to depletion of HDL-C^[43].

Other researchers showed similar results in experimental and clinical studies^[44,45] and they found a reduction in the oxidized LDL level in LA treated group.

The mechanism by which LA improves the dyslipidemia is still unclear. One of the actions which might attribute to this

finding is by decreasing the non-esterified fatty acid levels. The mechanism of action is also believed to be through the controlling activities of enzymes that involves in lipid metabolism. LA was reported to reduce 3-hydroxy-3-methyl glutaryl CoA (HMG-CoA) reductase activity as well as increases the lipoprotein lipase and lecithin cholesterol acyl transferase (LCAT) activities^[46].

Dyslipidemia is a primary risk factor for atherosclerosis development in general population and diabetic patients^[47]. Epidemiological surveys had shown that total cholesterol, triglyceride, LDL-C and HDL-C levels were useful in the assessment of cardiovascular risk in diabetic patients^[48].

In this work, non significant reduction in atherogenic index toward the control value was observed in LA-treated group. This reduction may be mainly due to the non significant reduction in serum total cholesterol rather than elevation in serum HDL-C level. Consequently, together with the reduction in serum TG, the risk for atherosclerosis could be diminished.

It is well documented that chronic hyperglycemia, a hallmark of the diabetic state, is associated with enhanced formation of reactive oxygen species, advanced glycation end-products and lipid peroxidation products and is also frequently accompanied by decreased antioxidant defenses^[49].

Excessive production of free radicals (by mechanisms such as autoxidation of glucose, enhanced glycation and altered polyol pathway) leads to the damage of proteins, lipids and DNA^[50].

Results of the present study showed significant reductions in serum levels of SOD, GSH and vitamin C, while serum uric acid level was not changed significantly in diabetic group, compared to control group. However, oral administration of LA improved significantly the different antioxidant defense systems in these patients.

The reports about the SOD activity in diabetes mellitus are controversial, with some authors reporting no change in SOD activity^[51,52] while others reported increased activity^[53,54]. There are also reports of decreased SOD activity in diabetic patients.

Because LA is lipid soluble, it is highly effective at reducing free radicals, including lipid peroxide, in cellular membrane. Because LA is also water soluble, it is able to gain access to the cytosol, where it effectively scavenges free radicals, capacity to regenerate endogenous antioxidants (such as vitamins C and E) and repair of oxidized proteins^[50]. It is also said that LA offer advantages over other antioxidants as it increases the level of reduced glutathione not only by regenerating the existing glutathione, but also by increasing its *de novo* synthesis^[55].

The increase in lipid peroxidation and the decline in antioxidant defense may appear early in type 2 diabetic patients, before the development of secondary complications and might play an important role in the initiation and progression of diabetic complications^[56].

Diabetic group revealed significant reduction in serum NO, compared to control group. Administration of LA returned the serum level of NO to the control value. The elasticity of the blood vessel wall is regulated by NO, a gas produced by

endothelial nitric oxide synthase (eNOS)^[40]. Several studies suggest that NO production is reduced in diabetes and that the decrease of NO may be related to the pathogenesis of diabetic endothelial damage^[57]. Cho^[58] reported that oxidative stress may reduce the bioavailability of NO, yielding low NO levels, so the reduction of serum NO in this study could be explained by the oxidative stress in diabetic patients. Again, Du *et al.*,^[59] illustrated that incubation of endothelial cells with LA protected the cultured cells against oxidative stress induced by hyperglycemia. Furthermore, in diabetic animal models, LA has been demonstrated to have beneficial effects on vascular and endothelial function as a result of its antioxidant potency^[60]. From all of the above, LA appears to have a potent beneficial role in addition to its role in management of some diabetic complications.

Significant reductions in serum iron and TIBC in addition to transferrin saturation in diabetic group were observed, compared to control group. Administration of LA improved iron level toward the control level.

Kayali *et al.*,^[61] reported that LA supplementation was associated with an increase in serum iron levels in aged rates.

The reduction in serum iron level in diabetic group may be due to decreased iron absorption as a result of decreased vitamin C which reduced dietary Fe⁺³ into the absorbed form Fe⁺²^[5]. Consequently, the improvement in antioxidants levels after LA administration leads to the elevation of serum iron level.

Moreover, the reduction of serum iron level in diabetic patients may be due to the increased insulin level which stimulates cellular iron uptake through increased transferrin receptor externalization^[62]. Lipoic acid administration improved the insulin sensitivity which leads to elevation in the iron level.

Davis *et al.*,^[63] demonstrated that iron-deficiency in female diabetic patient was associated with a marked rise in Hb A_{1c} levels. Tarim *et al.*,^[64] reported that iron supplementation for 3 months to diabetic patients decreased blood level of Hb A_{1c}. In addition, lipid peroxides play a role in hemoglobin glycation and antioxidants can inhibit the formation of glycated hemoglobin^[65,66]. From all of the above, the improvement in Hb A_{1c} may be the cause of serum iron elevation to the control value.

CONCLUSION

α - Lipoic acid improved the insulin resistance and oxidative stress in type 2 diabetics by increasing antioxidants. In addition, LA ameliorates the disturbance noticed in serum iron.

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