American Journal of Pharmacology and Toxicology 5 (3): 152-158, 2010
ISSN 1557-4962

α-Lipoic Acid Therapy Modulates Serum Levels of Some Trace Elements and Antioxidants in Type 2 Diabetic Patients

[1]Salwa Kamal El-Nabarawy, [2]Mona Abd El-Gelel Mohamed,
[3]Mohamed Mahmoud Ahmed and [4]Ghada Hany El-Arabi
[1]Department of Zoology, [2] Biochemistry Division, Chemistry Department,
Faculty of Science, Al-Azhar University, Egypt
[3]Department of Internal Medicine, National Research Center, Cairo, Egypt
[4]El-Tawfik Military Clinics, Cairo, Egypt

Abstract: Problem statement: The metabolism of several trace elements has been reported to alter in diabetes mellitus. The disturbance of their serum levels may have a role in the pathogenesis and progress of this disease. In addition, the reports about the status of antioxidants and antioxidant enzymes in diabetic patients are very contradictory. Though α-Lipoic Acid (LA) has long been touted as antioxidant, it has also been shown to improve glucose handling. **Approach:** The present study aimed to investigate the effect of exogenous supplementation of LA (600mg day^{-1}) on the serum levels of some antioxidants (Superoxide Dismutase (SOD), Glutathione (GSH) and vitamin C) and trace elements (Zn, Se, Cu and Co) in type 2 diabetic patients. The aim of this study was also extended to study the association of these parameters with the glycemic status. The studied participants were categorized into 3 groups (15 each): Control group: Non diabetic healthy volunteers. Diabetic group: Type 2 diabetic patients. Lipoic acid treated group: Type 2 diabetic patients received α-lipoic acid for 2 months in a daily oral dose of 600 mg. All participants were subjected to full history taking and complete clinical examination during the experimental period. Fasting serum samples were taken for determination of glucose, insulin, lipid profile and antioxidants as well as some trace elements. Homeostasis Model Assessment (HOMA) was also calculated as a marker of insulin resistance. **Results:** The results revealed that LA improved insulin resistance present in diabetic patients. The effects of oral treatment with LA were pronounced by improvement of serum levels of antioxidants (SOD and GSH, $p<0.001$, while vitamin C, $p<0.02$), Zn and Se (amounted 24.17 and 23.24%, respectively), compared to diabetic group. Oral treatment with LA produced strong positive correlations between serum levels of fasting glucose and both SOD and Se ($r = 0.8$). In addition, LA converted the negative correlation between SOD and HOMA index in diabetic patients into strong positive correlation. **Conclusion:** This study suggests that oral administration of LA (600mg day^{-1}) for 2 months to type 2 diabetic patients alleviated the insulin resistance, oxidative stress and lipid profile abnormalities. In addition, LA returned the serum levels of Zn and Se to the normal values.

Key words: α-Lipoic acid, trace elements, superoxide dismutase, glutathione, Diabetes Mellitus (DM) and Homeostasis Model Assessment (HOMA)

INTRODUCTION

Diabetes Mellitus (DM) is one of the most common chronic diseases in nearly all countries and continues to increase in numbers and significance (Shaw *et al*., 2010). Diabetes is not simply a disorder of glucose homeostasis but is also accompanied by various degenerative manifestations such as accelerated aging, cardiovascular disease and microvascular lesions leading to retinopathy, neuropathy and glomerulopathy. These events may be related to the hyperproduction of free radicals and to a dysfunction of biological antioxidant systems such as low enzyme activity or deficient micronutrient status (Faure *et al*., 1993).

Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals, such as super oxide (O_2•-), hydrogen peroxide (H_2O_2) and hydroxide (OH•-) radicals and deficiency in antioxidant defense mechanisms. Increased non-enzymatic and autooxidative glycosylation is one of the

Corresponding Author: Mona Abd El-Gelel Mohamed, Biochemistry Division, Chemistry Department, Faculty of Science, Al-Azhar University, Naser City, Cairo, Egypt

possible mechanisms that contribute to the formation of free radicals and free radical-induced lipid peroxidation in diabetes mellitus (Hunt *et al*., 1990).

The reports about the status of antioxidants and antioxidant enzymes in diabetic patients are very contradictory, both increases and decreases of antioxidant activity have been reported (Soliman, 2008).

There is accumulating evidence that the metabolism of several trace elements is altered in DM and these nutrients might have specific roles in the pathogenesis and progress of this disease (Kamal *et al*., 2009).

Alpha-lipoic acid (LA, thioctic acid) is synthesized in liver and other tissues, acts as a co-factor in a multi-enzyme dehydrogenase complex. There is growing evidence that orally supplied lipoic acid may not be used as a metabolic cofactor but instead, elicits a unique set of biochemical activities with potential pharmaco-therapeutic value against a host of patho-physiologic insults. It is used clinically for treatment of diabetic neuropathy (Shay *et al*., 2009).

While beneficial roles for LA and its reduced form (DHLA) in augmenting antioxidant defenses have been well characterized, much less is known about their potential role in modulating trace element status in vivo. The present study was undertaken to investigate the effect of exogenous supplementation of Lipoic acid on the serum levels of some trace elements (Zn, Cu, Co and Se) and some antioxidants in type 2 diabetic patients. The aim of this study also was extended to study the association of these parameters with the glycemic status.

MATERIALS AND METHODS

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 40-55 years (46.6±7.94) with Body Mass Index (BMI) 30.02±1.85 Kg m^{-2}. In addition, 15 age and sex matched healthy volunteers (BMI 28.74±2.43 Kg m^{-2}) were included. Written consent was obtained from all participants who were fully informed of the purpose of the study. All participants were subjected to full history taking and complete clinical examination throughout the study which extended for 2 months.

The diagnosis of type 2 DM was based on the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (2002). Duration of DM ranged from 3-8 years (4.13±1.41). ALL patients were on metformin in a dose 2000 mg day^{-1} and glimepiride in a dose of 2-3 mg day^{-1}. Diabetic patients were not taking any medicines other than oral anti-diabetic drugs. Special stress was given to vitamin, 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 diseases. Patients with diabetic 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, age and sex matched apparently healthy volunteers. Diabetic group (Diabetic)/comprising 15 type 2 diabetic patients. Lipoic acid treated group (Diabetic + LA)/diabetic patients (n = 15) who were convinced to receive α-lipoic acid for 2 months in a daily oral dose of 600 mg.

From all subjects, 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 level was assayed by glucose oxidase method (Barham and Trinder, 1972). Levels of serum insulin were assayed by ELISA (Dhahir *et al*., 1992). Homeostasis Model Assessment (HOMA), which correlates positively with insulin-resistance, was calculated according to Mahfouz *et al*. (2010). Triacylglycerol (TG) was assayed by peroxidase coupled method of Jacobs and Van Denmark (1960) and High Density Lipoprotein-Cholesterol (HDL-C) by the enzymatic method after precipitation of other lipoproteins with $MgCl_2$ and dextran sulphate according to the method of Finley (1978). Low Density Lipoprotein-Cholesterol (LDL-C) was assayed by the enzymatic method after precipitation of the LDL fraction by polyvinyl sulphate according to the method of Kerscher *et al*. (1985) while Very Low Density Lipoprotein-Cholesterol (VLDL-C) was calculated according to Freidwald *et al*. (1972). Serum total superoxide dismutase activity (SOD) was determined according to the method of Maier and Chan (2002), while serum glutathione was determined by enzymatic colorimetric method described by Onyango and Khan (2006). Determination of the serum level of vitamin C was performed according to the method of Jagota and Dani (1982). Serum levels of trace elements (Zn, Cu, Co and Se) were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce, Japan).

Statistical analysis: All results were expressed as the mean ± 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. Spearman's correlation test was used for correlating non-parametric variables. For all tests a probability (p) less than 0.05 was considered significant (Dawson and Trapp, 2001).

RESULTS

Data presented in Table 1, illustrate that glucose homeostatic parameters (fasting serum glucose, insulin and HOMA) studied in both groups; diabetic untreated and diabetic treated with lipoic acid, were significantly ($p<0.0001$) elevated, compared to control group. Non significant improvement in serum level of glucose was observed in LA treated group, compared to diabetic group. However, administration of lipoic acid improved significantly the values of insulin and HOMA index ($p<0.001$ and 0.02, respectively), compared to diabetic untreated group.

The studied lipid profile parameters showed significant elevations in the serum levels of 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. On the other hand, significant reductions ($p<0.03$) in the serum levels of TG and VLDL-c were observed in Lipoic treated group, compared to diabetic untreated group.

The levels of serum antioxidants and trace elements are shown in Table 2. Significant reductions were noticed in the serum levels of all the antioxidant parameters ($p<0.0001$) in diabetic group, compared to control group. Lipoic acid group showed no 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. Significant reduction in the serum level of Zn ($p<0.0001$) was observed in diabetic group, while serum level of Se was no significant compared to control group. Treatment with Lipoic acid improved significantly the serum levels of Zn and Se (amounted 24.17 and 23.24%, respectively), compared to untreated diabetic group.

In regard to serum levels of Cu and Co, lipoic acid administration did not affect their levels. Cu was significantly elevated ($p<0.0001$) while Co level did not change in both diabetic groups as compared to control group.

Table 1: Serum levels of glucose homeostatic parameters, TG and lipoproteins-C in the different studied groups (Means ± SE)

Parameters	Control	Diabetic	Diabetic + LA
Glucose (mg dL^{-1})	76.20±1.19	167.00±14.60[a]	163.20±9.99[a]
Insulin (μIU mL^{-1})	7.03±0.24	20.16±0.44[a]	14.00±0.41[a,b]
HOMA index	1.33±0.05	8.40±0.80[a]	5.69±0.40[a,b]
TG (mg dL^{-1})	137.38±5.20	260.67±22.99[a]	200.40±5.92[a,b]
HDL-C (mg dL^{-1})	54.60 ±4.25	40.80±1.11[a]	42.20±0.93[a]
LDL-C (mg dL^{-1})	107.14±10.62	151.67±4.44[a]	141.32±5.06[a]
VLDL-C (mg dL^{-1})	27.48±1.04	52.13±4.60[a]	40.08±1.18[a,b]

[a]: Significant and control; [b]: Significant and diabetic

Table 2: Serum levels of some antioxidants and trace elements in the different studied groups (Means ± SE)

Parameters	Control	Diabetic	Diabetic + LA
SOD (μ mL^{-1})	13.09±0.83	5.68±0.19[a]	13.48±0.70[b]
GSH (pmol)	27.93±1.20	18.93±0.77[a]	26.20±1.14[b]
Vit. C (μg mL^{-1})	4.90±0.46	2.50±0.17[a]	3.54±0.29[a,b]
Zn (μg L^{-1})	143.73±2.95	116.13±3.72[a]	144.20±6.40[b]
Se (μg L^{-1})	16.40±1.05	13.60±1.25	16.76±0.86[b]
Cu (mg L^{-1})	2.37±0.12	3.61±0.16[a]	3.24±0.13[a]
Co (μg L^{-1})	3.48±0.96	2.96±0.19	3.34±0.20

[a]: Significant and control; [b]: Significant and diabetic

Table 3: Correlations between different parameters in diabetic untreated and LA treated groups

Parameters	Group	r	P
Glucose and SOD	LA treated	0.80	0.001
Glucose and Se	LA treated	0.80	0.001
HOMA and SOD	diabetic untreated	-0.53	0.040
HOMA and SOD	LA treated	0.61	0.010

Administration of α-lipoic acid produced strong positive correlations between serum level of fasting glucose and both SOD and Se ($r = 0.8$). In addition, significant negative correlation was observed between HOMA index and serum level of SOD ($r = -0.53$) in diabetic group, which changed into positive correlation ($r = 0.61$) after administration of lipoic acid (Table 3).

DISCUSSION

Under fasting conditions, glucose homeostasis is maintained by the balance between systemic glucose production and total-body glucose utilization. The increase in glucose production can be largely attributed to an elevation in the gluconeogenesis rate and appears as the main cause of fasting hyperglycemia, which is particularly evident in a certain percentage of diabetic patients (Khamaisi *et al*., 1999).

The present results showed a state of insulin resistance in diabetic patients, as demonstrated by significant elevation of serum glucose, insulin levels and higher value of HOMA index as compared with control. Administration of α-lipoic acid improved significantly this insulin resistance as observed by the reduction in insulin and HOMA values. These results agree with those of Jacob *et al*. (1995); Jacob *et al*. (1996) and Konrad *et al*. (1999).

Lipoic acid was found to enhance expression of the Insulin Receptor Substrate-1 (IRS-1) protein in muscle of obese Zucker rats (Saengsirisuwan *et al*., 2004). In addition, LA proposed to recruit GLUT4 in skeletal muscles from its storage site, so that glucose uptake is stimulated by the local increase in transporter (Shay *et al*., 2009). Unchanged serum glucose level in LA group may be due to insufficient time duration of the experiment.

Dyslipidemia, hallmarked by low plasma HDL-C and high LDL-C and triacylglycerol levels, is common in patients with diabetes mellitus (Refaie *et al*., 2005). 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.

The present study revealed pronounced elevations in the serum levels of the lipid profile except HDL-C which showed significant reduction in diabetic group. Type 2 diabetes is usually associated with higher concentrations of TC and TG and lower concentrations of HDL-C. Insulin resistance is likely underlies these changes (Garvey *et al*., 2003; Rosenson, 2006).

In this study, the serum level of VLDL-C was much elevated in diabetic group in relation to control group. Krauss and Siri (2004) 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 cholesterol and to depletion of HDL cholesterol (Duell *et al*., 1991).

In this study, although oral administration of LA improved significantly the serum levels of TG and VLDL-C toward the control values, the levels of LDL-C and HDL-C showed no significant improvement. The mechanism by which ALA 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. ALA was reported to reduce HMG-CoA reductase activities as well as increases the lipoprotein lipase and Lecithin Cholesterol Acyl Transferase (LCAT) activities (Siti *et al*., 2007).

Free radical production can be enhanced during hyperinsulinemia and hyperglycemia by mechanisms such as autoxidation of glucose, enhanced glycationand altered polyol pathway (Mahfouz *et al*., 2009).

In the present study, there was a significant decrease in serum levels of SOD, GSH and vitamin C in diabetic group, compared to control. However, oral administration of LA improved significantly the antioxidant defense mechanism in these patients.

Lipoic acid is a unique antioxidant because it has beneficial effects on fuel metabolism and also an essential cofactor of mitochondrial respiratory enzymes, including the pyruvate dehydrogenase complex (Siti *et al*., 2008). It is said that LA offer advantages over other antioxidants as it increases the level of reduced glutathione (Osfor *et al*., 2010) and can also regenerate other antioxidants such as vitamin C and E (Cakatay *et al*., 2005).

The reports about the SOD activity in diabetes mellitus are controversial, with some authors reporting no change in SOD activity (Ramazan *et al*., 2000 and Kesavulu *et al.*, 2000) while others reported increased activity (Maritim *et al.*, 2003; Sailaja *et al*., 2003). There are also reports of decreased SOD activity in diabetic patients. A decreased level of GSH indicates decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid peroxidation processes in these patients (Soliman, 2008).

The improvement in SOD level in our diabetic patients may be due to the improvement in Zn level since extracellular SOD is Cu-Zn dependent (Zelko *et al*., 2002), since zinc ions stabilize the apoprotein whereas Cu is a prosthetic group of the active site of SOD.

The increase in lipid peroxidation and the decline in antioxidant defense may appear early in type 2 DM patients, before the development of secondary complications and might play an important role in the initiation and progression of diabetic complications (Soliman, 2008). LA converted the negative correlation between SOD and both HOMA index and fasting glucose in diabetic patients into a positive correlation in the diabetic treated group. This means that LA appears to improve the antioxidant capacity in face of the oxidative stress induced by insulin resistance in type 2 DM. From all of the above, LA appears to have a potent therapeutic role in addition to its role in management of diabetic neuropathy in protection of diabetic complications due to oxidative stress.

Zinc and selenium may have utility in the treatment of the complex disorders in type 2 diabetes and may help in the control of blood glucose and lipids, thus preventing or delaying serious clinical events (Kamal *et al*., 2009).

Results of the present work revealed significant reduction in serum level of Zn in diabetic group compared to non-diabetics as reported earlier (Zheng *et al*., 2008; Kazi *et al*., 2008;

Viktorínová *et al*., 2009; Hussain *et al*., 2009). However, comparable serum levels of zinc in the type 2 diabetic patients and normal subjects have been reported elsewhere (Walter *et al*., 1991). Nevertheless most studies have found an increased urinary zinc loss in these patients (Hagglof *et al*., 1983; Faure *et al*., 1993).

In this study, treatment with LA returned the serum levels of Zn and Se to the normal control values. Zinc results are in line with those reported by Kayali *et al*. (2007), while for Se, as a matter of fact, there are no studies in the literature about the effect of LA on serum Se. The normalization of serum levels of Zn and Se may be due to the improvement in the insulin sensitivity as indicated by the lowered value of HOMA. Interestingly, the normalization of the Zn and Se was accompanied by a decrease in lipid abnormalities which could reflect the role of these trace elements as biological antioxidants.

The redox chemistry of Cu makes this both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical. Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications (Hussain *et al*., 2009).

The present study revealed significant elevation in the serum level of Cu in diabetic group. Other studies found that in diabetic patients, circulating copper concentrations were not different (Bo *et al*., 2008; Hussain *et al*., 2009) or were greater (Zargar *et al*., 1998 and Abu-seif and Youssef, 2004). Treatment with LA did not reduce the elevated serum level. On the contrary, Osfor *et al*. (2010) indicated that injecting Cu- intoxicated rats with α-LA significantly reduced serum copper.

The present study did not find correlation between copper and any of the studied parameters. Consequently, the increased copper levels found in diabetics in our study may merit further investigation of the relationship between copper and type 2 DM.

Although diabetic untreated group showed reduction in the serum level of Co which improved and returned to the control value in lipoic acid treated group, but these alterations were not statistically significant. Serum Cu and Co concentrations are perhaps not the best index of total body Cu and Co.

CONCLUSION

It is concluded that oral administration of LA to type 2 DM alleviated the glycemic status and oxidative stress in addition to Zn and Se serum levels. Consequently, LA may delay the diabetic complications.

REFERENCES

Abu-Seif, M.A. and A.A. Youssef, 2004. Evaluation of some biochemical changes in diabetic patients. Clin. Chem. Acta., 16: 161-70. DOI: 10.1016/j.cccn.2004.03.030

Barham, D. and P. Trinder, 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, 97: 142-145. PMID: 5037807

Bo, S., M. Durazzo, R. Gambino, C. Berutti and N. Milanesio *et al*., 2008. Associations of dietary and serum copper with inflammation, oxidative stress and metabolic variables in adults. J. Nutr., 138: 305-10. PMID: 18203896

Cakatay, U., R. Kayali, A. Sivas and F. Tekeli, 2005. Prooxidant activities of alpha-lipoic acid on oxidative protein damage in the aging rat heart muscle. Arch. Gerontol. Geriatr., 40: 231-40. DOI: 10.1016/j.archger.2004.09.001

Dawson, B., R.G. Trapp, 2001. Basic and Clinical Biostatistics. 3rd Edn., PBL. Lange Medical Books/McGraw-Hill, USA., ISBN: 0838505104, pp: 399.

Dhahir, F.J., D.B. Cook and C.H. Self, 1992. Amplified enzyme-linked immunoassay of human proinsulin in serum. Clin. Chem., 38: 227-32. PMID: 1541005

Duell, P.B., J.F. Oram and E.L. Bierman, 1991. Nonenzymatic glycosylation of HDL and impaired HDL-receptor mediated cholesterol efflux. Diabetes, 40: 377-384. PMID: 1847886

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care, 26: 5-20. PMID: 12502614

Faure, P., P. Cortlce, M.J. Richard, J. Arnaud and C. Coudray *et al*., 1993. Lipid Peroxidation and Trace Element Status in Diabetic Ketotic Patients: Influence of Insulin Therapy. Clin. Chem., 39: 789-793. URL: http://www.clinchem.org/cgi/content/abstract/39/5/789

Finley, P.R., R.B. Schifman, R.J. Williams and D.A. Lichhti, 1978. Cholesterol in high-density lipoprotein: Use of Mg2+/dextran sulphate in its enzymatic measurement. Clin. Chem., 24: 931-3. PMID: 207463

Friedewald, W.T., R.J. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of the preparative ultracentrifuge. Clin. Chem., 18: 499-502. PMID: 4337382

Garvey, W.T., S. Kwon, D. Zheng, S. Shaughnessy and P. Wallace *et al*., 2003. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes, 52: 453-62. DOI: 10.2337/diabetes.52.2.453

Hagglof, B., G. Hailman, G. Hoingren, J. Ludvigson and A. Falkmer, 1983. Prospective and retrospective studies of zinc concentrations in serum, blood, clots, hair and urine in young patients with insulin dependent diabetes mellitus. Acta. Endocrinol., 102: 88-95. PMID: 6337453

Hunt, J.V., C.C. Smith and S.P. Wolff, 1990. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes, 39: 1420-4. PMID: 2227114

Hussain, F., M.A. Maan, M.A. Sheikh, H. Nawaz and A. Jamil, 2009. Trace elements status in type 2 diabetes. Bangladesh J. Med. Sci., 8: 52-56. URL: http://lib-journals5.lib.sfu.ca:8108/index.php/BJMS/article/viewFile/3983/3259

Jacob, S., E.J. Henriksen, A.L. Shiemann, I. Simon and D.E. Clancy *et al*., 1995. Enhancement of glucose disposal in patients with type 2 diabetes by alpha-lipoic acid. Arzneimittelforschung, 45: 872-74. PMID: 7575750

Jacob, S., R.S. Streeper, D.I. Fogt, J.Y. Hokama and H.J. Tritschler *et al*., 1996. The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. Diabetes, 45: 1024-29. PMID: 8690147

Jacobs, N.J. and P.J. Van Denmark, 1960. The purification and properties of the alpha-glycerophosphate-oxidizing enzyme of Streptococcus faecalis 10 C_1. Arch. Biochem. Biophys., 88: 250-55. PMID: 14406376

Jagota, S.K. and H.M. Dani, 1982. A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal. Biochem., 127: 178-182. DOI: 10.1016/0003-2697(82)90162-2

Kamal, M., M. Salem, N. Kholousi and K. Ashmawy, 2009. Evaluation of trace elements and malondialdehyde levels in type II diabetes mellitus. Diabetes and Metabolic Syndrome: Clin. Res. Rev., 3: 214-8. DOI: 10.1016/j.dsx.2009.07.007

Kayali, R., U. Cakatay, A.R. Kiziler and B. Aydemir, 2007. Effect of alpha-lipoic acid supplementation on trace element levels in serum and postmitotic tissue in aged rats. Med. Chem., 3: 297-300. PMID: 17504203

Kazi, T.G., H.I. Afridi, N. Kazi, M.K. Jamali and M.B. Arain *et al*., 2008. Copper, chromium, manganese, iron, nickeland zinc levels in biological samples of diabetes mellitus patients. Biol. Trace Elem. Res., 122: 1-18. DOI: 10.1007/s12011-007-8062-y

Kerscher, L., S. Schiefer and B. Draeger, 1985. Precipitation methods for the determination of LDL-cholesterol. Clin. Biochem., 18: 118-125. PMID: 3893795

Kesavulu, M.M., R. Giri, R.B. Kameswara and C. Apparao, 2000. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes Metab., 26: 387-92. PMID: 11119018

Khamaisi, M., A. Rudich, R. Potashnik, H.J. Tritschler and A. Gutrnan *et al*., 1999. Lipoic acid acutely induces hypoglycemia in fasting non diabetic and diabetic rats. Metabolism, 48: 504-10. DOI: 10.1016/S0026-0495(99)90112-9

Konrad, T., P. Vicini, K. Kusterer, A. Hoflich and A. Assakhani *et al*., 1999. Alpha-lipoic acid treatment decreases serum lactate and pyruvate concentrations and improves glucose effectiveness in lean and obese patients with type 2 diabetes. Diabetes Care, 22: 280-87. PMID: 10333946

Krauss, R.M. and P.W. Siri, 2004. Dyslipidemia in type 2 diabetes. Med. Clin. North. Am., 88: 897-909. URL: http://www.medical.theclinics.com/issues

Mahfouz, M.H., H.M. Ghanem and M.A. Mohamed, 2009. Therapeutic effect of L-carnitine on sialic acid, soluble Fas (sFas) and other biochemical variables in hyperinsulinemic rats. Life Sci. J., 6: 76-82. ISSN: 1097-8135

Mahfouz, M.H., H.M. Ghanem and M.A. Mohamed, 2010. Modulation of insulin receptor substrate-1 and some inflammatory variables in hyperinsulinemic rats treated with cinnamon extract. Am. J. Biochem. Biotech., 6: 11-18. ISSN: 1553-3468

Maier, C.M. and P.H. Chan, 2002. Role of superoxide dismutases in oxdative damage and neurodegenerative disorders. Neuroscientist, 8: 323-334. PMID: 12194501

Maritim, A.C., R.A. Sanders and J.B. Watkins, 2003. Diabetes, oxidative stress and antioxidants: A Rev. J. Biochem. Mol. Toxicol., 17: 24-38. PMID: 12616644

Onyango, I.G. and S.M. Khan, 2006. Oxidative stress, mitochondrial dysfunctionand stress signaling in Alzheimer's disease. Curr. Alzheimer Res., 3: 339-349. PMID: 17017864

Osfor, M.M., H. Ibrahim, Y.A. Mohamed, S.M. Ahmed and A.S. Abd El Azeem *et al.,* 2010. Effect of alpha lipoic acid and vitamin E on heavy metals intoxication in male albino rats. J. Am. Sci., 6: 56-63. ISSN: 1545-1003

Ramazan, M., E. lu, H. Sahin, H. Dulger and E. Algun, 2000. The effect of dietary treatment on erythrocyte lipid peroxidation, superoxide dismutase, glutathione peroxidase and serum lipid peroxidation in patients with type 2 diabetes mellitus. Clin. Biochem., 33: 669-74. DOI: 10.1016/S0009-9120(00)00190-9

Refaie, F.M., A.Y. Esmat, S.M. Abdel Gawad, A.M. Ibrahim and M.A. Mohamed, 2005. The antihyperlipidemic activities of 4(3H) quinazolinone and two halogenated derivatives in rats. Lipids in Health Dis., 4: 22-33. DOI: 10.1186/1476-511X-4-22

Rosenson, R.S., 2005. HDL-C and the diabetic patient: Target for therapeutic intervention? Diabetes Res. Clin. Pract., 68: 36-42. PMID: 15953506

Saengsirisuwan, V., F.R. Perez, J.A. Sloniger, T. Maier and E.J. Henriksen, 2004. Interactions of exercise training and alpha-lipoic acid on insulin signaling in skeletal muscle of obese Zucker rats. Am. J. Physiol. Endocrinol. Metab., 287: 529-36. PMID: 15068957

Sailaja, Y.R., R. Baskar and D. Saralakumari, 2003. The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. Free Radic. Biol. Med., 35: 133-39. PMID: 12853069

Shaw, J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 4-14. DOI: 10.1016/j.diabres.2009.10.007

Shay, K.P., R.F. Moreau, E.J. Smith, A.R. Smith and T.M. Hagen, 2009. Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. Biochem. Biophys. Acta, 1790: 1149-60. DOI: 10.1016/j.bbagen.2009.07.026

Siti, B.B., O. Khairul, W.W. Nazaimoon, O. Faizah and R.L. Santhana *et al*., 2008. Alpha-lipoic acid reduces plasma glucose and lipid and the ultra-microscopic vascular changes in streptozotocin-induced diabetic rats. Annals of Microscopy, 8: 58-65. ISSN: 0219-2209

Siti, B.B., P.K. Kwa, Y.S. Monica, O. Khairul and A.B. Mokhtar *et al*., 2007. Alpha-lipoic acid prevents pancreatic islet cells damage and dyslipidemia in streptozotocin-induced diabetic rats. Malaysian J. Med. Sci., 14: 47-53. ISSN: 13-94-195X

Soliman, G.Z., 2008, Blood lipid peroxidation (superoxide dismutase, malondialdehyde, glutathione) levels in Egyptian type 2 diabetic patients. Singapore Med. J., 49: 129-33. PMID: 18301840

Viktorínová, A., E. Toserová, M. Krizko and Z. Duracková, 2009. Altered metabolism of copper, zincand magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus. Metabolism, 58: 1477-1482. PMID: 19592053

Walter, R.M., J.Y. Uriu-Hare, K.L. Olin, M.H. Oster and B.D. Anawalt *et al*., 1991. Copper, zinc, manganese and magnesium status and complications of diabetes mellitus. Diab. Care, 11: 1050-56. PMID: 1797486

Zargar, A.H., N.A. Shah, S.R. Masoodi, B.A. Laway and F.A. Dar *et al*., 1998. Copper, zincand magnesium levels in non-insulin dependent diabetes mellitus. Postgrad. Med. J., 74: 665-68. PMCID: PMC2431594

Zelko, I.N., T.J. Mariani and R.J. Folz, 2002. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolutionand expression. Free Rad. Biol. Med., 33: 337-49. PMID: 12126755

Zheng, Y., X.K. Li, Y. Wang and L. Cai, 2008. The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators. Hemoglobin, 32: 135-45. PMID: 18274991