

ORIGINAL ARTICLE

EFFECTS OF ALPHA LIPOIC ACID ON OXIDATIVE STRESS AND CONTRACTILE FUNCTIONS OF FAST MUSCLES IN TYPE 2 DIABETIC MALE SPRAGUE DAWLEY RATS

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Background: Metabolic derangements and oxidative stress due to Type 2 Diabetes Mellitus (T2DM) has deleterious effects on skeletal muscle contractile functions. This study aimed to see the effects of alpha lipoic acid (ALA) on oxidative stress and contractile functions of fast muscles in type 2 diabetic male Sprague Dawley rats. **Methods:** This quasi experimental study was conducted at Department of Physiology, CMH Multan Institute of Medical Sciences, Multan from Sep 2022 to Feb 2023. Ninety adult Sprague-Dawley rats were randomly divided into 3 equal groups (n=30). Group A (control group) Group B (Diabetic group) and Group C (ALA-treated group). Diabetes was induced in group B and C by injecting Streptozocin (35 mg/Kg body weight) intraperitoneally in the lower-right quadrant of the abdomen after 2 weeks. After 4 weeks, extensor digitorum longus (EDL) muscles were dissected and contractile functions assessed through iWorx data acquisition unit. Serum Malondialdehyde (MDA) and plasma glucose levels were estimated through cardiac blood sampling. **Results:** ALA group showed improvement in maximum fused tetanic tension, fatigue and recovery from fatigue protocol as compare to the diabetic group. No significant difference among all the groups was found in maximum tension and time to relax to peak twitch tension. Serum MDA levels were found significantly decreased in ALA group as compared to the diabetic group. **Conclusion:** ALA supplementation decreases oxidative stress which improves contractile force and delays fatigue in fast muscles of diabetic rats.

Keywords: Alpha lipoic acid, contractile function, malondialdehyde, muscle, type 2 diabetes mellitus

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INTRODUCTION

According to World Health Organization (WHO) approximately 462 million people are affected worldwide from Type 2 diabetes mellitus (T2DM) currently, and by 2035 this number may rise to 592 million.¹ Muscle fibres have been classified into two distinct categories: type I (slow twitch, oxidative) and type II (fast twitch, glycolytic).² Skeletal muscles utilize muscle glycogen and plasma glucose by oxidation for optimal adenosine triphosphate (ATP) generation for daily activities and during high intensity exercise.³ In T2DM, decreased insulin sensitivity/resistance results in mitochondrial dysfunction with defective oxidative phosphorylation, decreased content and rate of glycogen synthesis, decreased glucose oxidation due to decreased pyruvate dehydrogenase (PDH) activity resulting in increased dependence on alternate sources of energy via accelerated lipid and protein catabolism. Consequently, there is increased plasma concentration of triglycerides and free fatty acids (FFAs)⁴ which lead to formation of superoxide anion, hydroxyl radical and other reactive oxygen species (ROS) like hydrogen peroxide which causes cytotoxic damage to different proteins in mitochondria which affect fast muscle fibres contractile functions by decreasing its oxidative capacity.⁵

Alpha-lipoic acid (ALA) plays a crucial role in

mitochondrial energy metabolism as a cofactor for α -ketoacid dehydrogenases and functions as a potent antioxidant. It scavenges ROS, regenerates intracellular antioxidants like glutathione and vitamins C and E, and enhances insulin sensitivity. ALA stimulates glucose uptake by translocating GLUT4 transporters and activating AMP-activated protein kinase, promoting fatty acid oxidation.

In T2DM, increased oxidative stress and impaired glucose metabolism lead to contractile dysfunction in skeletal muscles, particularly fast-twitch fibres. ALA mitigates these effects by reducing oxidative stress, restoring glycogen synthase activity, and improving glucose uptake, thereby enhancing muscle contractile function and delaying fatigue. Alpha-lipoic acid is naturally synthesized enzymatically in the mitochondrion from octanoic acid. In addition to *de novo* synthesis yeast, animal liver, kidney, and spinach, broccoli, potatoes are good plant sources of ALA.⁶

Many international studies have proven that ALA corrects metabolic derangements and oxidative stress in T2DM however no study has been conducted regarding its effects on contractile functions of skeletal muscles. This study aimed to see the effects of alpha lipoic acid on oxidative stress and contractile functions of fast muscles in T2DM male Sprague Dawley rats.

METHODOLOGY

This experimental control trial was carried out at Physiology and Anatomy Departments of CMH Multan Institute of Medical Sciences, Multan from Sep 2022 to Feb 2023 after formal approval from ERC/IRB of the Institution. Ninety Sprague Dawley rats (adult male) were recruited for the study.

Male Sprague-Dawley rats (82±6 days old) and 245±60 grams average body weight were included in the study. Three groups (each having 30 rats) were formed by random distribution. The control group was fed on low fat normal diet and free access to water. Diabetic and ALA groups were fed on fat rich/high fat diet (HFD) for 14 days. (Table-1).

Table-1: Composition of rat's feed

Control group		Diabetic and ALA groups	
Ingredients	Weight (g/Kg)	Ingredients	Weight (g/Kg)
Wheat	275	NPD	385
Dried skimmed milk	285	Casein	315
Mollasen	5	Lard	265
Salt	15	Cholesterol	30
Cooking oil (mL/Kg)	50	Vitamin/mineral	70
Raw meat	150	L-cystine	3
Vitamins	10	Yeast mixture	5
Wheat brown	200	Sodium chloride	20

On 15th day, diabetes mellitus was induced by injecting Streptozocin (35 mg/Kg body weight) intraperitoneally in diabetic and ALA groups, while normal saline was injected in control group. On 21st day, development of T2DM in diabetic and ALA groups was confirmed (plasma glucose level >16.65 mmol/L) by measuring plasma glucose of all rats by tail vein sampling.⁷

Alpha lipoic acid (Thioctacid 600, AstaMedica, Germany) was injected intra-peritoneally (30 mg/Kg/day) for two weeks to ALA group, while normal saline was administered to other two groups.⁸

On 28th day, rats were euthanized by high dose of Ether. Samples of 3–5 mL blood was obtained and centrifuged for 15 minutes at 4,000 rpm at 4 °C. Serum was pipetted out and transferred into 1.5 mL labelled eppendorf tubes to assess biochemical parameters.

Serum malondialdehyde (MDA) levels were estimated using Rat MDA ELISA Kit, (Shanghai Crystal Day Biotech Co, Ltd.). Plasma glucose levels (PGLs) was estimated by Glucose kit (glucose oxidase method).

On 28th day, extensor digitorum longus muscle (EDL) was dissected and mounted on organ bath system of iWorx for animal physiology data acquisition unit containing 25 mL Krebs-Ringer bicarbonate buffer solution and supplied with 95% O₂ and 5% CO₂ continuously at fixed temperature of 30 °C.⁹ Force transducer of iWorx was used for muscle stimulation

with stimulation frequencies 5–110 Hz per second (with 3 minutes rest period between each stimulus) was used for measuring peak twitch tension (PTT), force-frequency relationship and relax time to 50% of the PTT. Maximum fused tetanic tension (MFTT) and recovery from fatigue was determined by stimulating muscle with optimum frequency for 1 minute with 5 seconds rest time in between.¹⁰

The Mean±SD was calculated using SPSS-23. ANOVA was applied to determine the statistically significant differences across the groups and followed by post hoc test, and $p \leq 0.05$ was considered significant.

RESULTS

Plasma glucose levels (PGLs) and body weight of all rats recorded on day one were within normal range. Diabetic and ALA rats groups successfully developed T2DM confirmed on 21st day of the study by measuring plasma glucose levels. As compared to control group, diabetic and ALA groups rats body weight was found to be increased due to fat rich high calorie diet. PGLs and BW of each rat was again measured at the end of study on 28th day and were found significantly high in the diabetic group, while the ALA group showed decrease levels compare to diabetic group (Table-2).

Serum MDA levels were 3.71±0.66 µmol/dL, 7.97±0.81 µmol/dL in diabetic group, and 4.45±0.72 µmol/dL in ALA group. Skeletal muscles parameters like TPTT ($p > 0.38$), MTT ($p > 0.15$), and relax time to 50% maximum twitch tension ($p > 0.29$) were insignificant among all groups. Significant difference was found among the groups in fatigue protocols like MFTT ($p < 0.02$), and tetanic tension measured after fatigue protocol ($p < 0.03$), (Table-3).

Diabetic group was significantly different from control and ALA after application of post hoc Tukey's test, and control and ALA groups had non-significant differences (Table-4).

Table-2: Body weight and plasma glucose levels in all groups at 1st, 21st, and 28th days (Mean±SD)

Groups	Days	Control	Diabetic	ALA
Body weight (g)	1	215.55±5.04	248.62±6.47	251.54±5.32
	21	256.63±7.40	269.70±8.35	268.70±7.70
	28	265.71±7.15	278.70±7.61	269.86±8.34
Plasma glucose (mmol/L)	1	5.85±0.34	5.84±0.30	5.87±0.34
	21	5.83±0.31	23.13±0.40	22.90±0.41
	28	5.91±0.32	23.90±0.47	10.55±0.45

Table-3: Skeletal muscle contractile parameters comparison using one-way ANOVA on 28th day

Variables	Group I (Control)	Group II (Diabetic)	Group III (ALA)	p
PTT (N/g)	0.34±0.06	0.31±0.07	0.40±0.021	0.15
TPTT (mSec)	20.83±1.53	22.05±1.23	21.15±1.54	<0.38
Relax time to 50% PTT	21.2±3.50	22.8±4.30	21.9±4.00	<0.9
MFTT (N/g)	3.98±0.07	3.93±0.09	3.97 ± 0.06	<0.02
MFTT after fatigue protocol (N/g)	1.83±0.05	1.79±0.05	1.82±0.06	<0.01

Table-4: Comparison of groups using post hoc Tukey's test (*p*-values)

Contractile parameters	Control vs Diabetic	Control vs ALA	Diabetic vs ALA
MFTT	0.01	0.55	0.04
MFTT after fatigue protocol	0.00	0.48	0.03

DISCUSSION

This study demonstrates that ALA supplementation reduces oxidative stress markers and improves certain aspects of skeletal muscle contractile function in diabetic rats. The findings align with previous research highlighting ALA's antioxidant properties and its role in improving glucose metabolism and mitochondrial function.^{6,11} The reduction in serum MDA levels observed in the ALA-treated group supports its role in mitigating oxidative stress, which is a key factor in T2DM-induced muscle dysfunction.⁵ Furthermore, the improvement in maximum fused tetanic tension and recovery from fatigue suggests that ALA may contribute to better muscle endurance by restoring glycogen synthase activity and ATP availability.⁴

A major strength of this study is its integration of metabolic and functional assessments of skeletal muscle performance. Previous studies have established ALA's role in improving insulin sensitivity and reducing oxidative damage.^{12,13} However, limited research has examined its direct impact on skeletal muscle contractility in diabetic models. This study adds to the existing body of knowledge by demonstrating that ALA may enhance muscle performance by reducing oxidative stress and improving glucose utilization in fast-twitch muscle fibres.

While the results support ALA's beneficial effects, claims regarding its ability to 'correct metabolic derangements' should be cautiously interpreted. The scope of this study was limited to biochemical markers of oxidative stress and muscle contractility, and it did not evaluate long-term metabolic adaptations or molecular mechanisms such as GLUT4 expression or mitochondrial function in detail. ALA supplementation reduced oxidative stress markers and improved muscle function in this model.

Malondialdehyde (MDA) is an end-product formed during increase lipid peroxidation which signifies cellular injury and oxidative stress. Serum MDA levels are used as biomarker of oxidative stress and were high in the diabetic group showing increase oxidative stress and damaging effects of reactive oxygen species (ROS). Serum MDA levels were decreased in the ALA group as compare to diabetic group signifying its antioxidant property.¹⁴ Zhang T *et al*¹⁵ documented the similar role of alpha-lipoic acid in protection against oxidative stress and apoptosis in rats with diabetic peripheral neuropathy by studying the mechanism of ALA through activated AMPK pathway.

The EDL muscle largely depends on glycogenolysis for ATP production because of abundant type II fast fibres.¹⁶ There were no significant differences in MITT, TPTT and relaxation time to 50% MITT among the groups as these skeletal muscles contractile functions depends on available ATPs and calcium ions in sarcoplasm, release of Ca⁺⁺ and transport back to sarcoplasmic reticulum via Ca⁺⁺ pump. Adequate amount of ATPs are available in the diabetic muscle sarcoplasm for a single muscle twitch and the activity of Ca⁺⁺ pump is also unaffected in early stages of T2DM.¹⁷

The MFTT and maximum muscle tension after fatigue protocol of the EDL muscle require large number of ATPs for tetanic contraction due its abundant fast fibres and is provided by already stored muscle glycogen. Glycogen synthase activity is halted in T2DM because of insulin resistance and oxidative stress causing significant reduction occurs in glycogen stores and ability to reuptake glucose.¹⁸ Therefore, maximum tension generated and muscle tension following fatigue protocol in diabetic muscles is significantly less than controls and ALA group. ALA decrease oxidative stress by reducing ROS and improved insulin sensitivity leading to restoration of glycogen synthase activity which improves glycogen storage and increase glucose reuptake thus providing large amount of ATPs to produce maximum tension and improved contraction force after fatigue protocol comparable to the controls muscles.¹⁹

Hong OK *et al*²⁰ has extensively explored the role of ALA in preservation of skeletal muscle mass (gastrocnemius) in type 2 diabetic OLETF rats similar to our settings which further establish the role ALA on skeletal muscles of diabetic rats. However his work was only on muscle mass and weight and not entirely on contractile functions.

Our study explored the beneficial effects of ALA on contractile functions of fast skeletal muscles and oxidative stress in diabetic rats and can be exogenously used as an adjunct therapy in treating T2DM induced cases of contractile dysfunction of skeletal muscles.

LIMITATIONS OF STUDY

The mechanism of action of ALA on glucose transporters and contractile proteins in EDL muscles in male diabetic rats could be explored in depth by immunohistochemistry *in vivo*.

CONCLUSION

Alpha-lipoic acid supplementation aid in restoring muscle contractile performance in T2DM by effectively reducing oxidative stress and restores metabolic equilibrium leading to improved muscle function, increased maximum tension, and enhanced recovery from fatigue.

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