ORIGINAL ARTICLE

Effects of Alpha Lipoic Acid Supplementation on Slow Skeletal Muscle Mass and Contractile Functions in Type 2 Diabetic Male Sprague Dawley Rats

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ABSTRACT

Objective: To see the Effects of Alpha-Lipoic Acid (ALA) supplementation on slow skeletal muscle mass and contractile functions in type 2 diabetic male Sprague Dawley rats.

Study Design: Quasi-experimental study.

Place and Duration of Study: The study was carried out at the Physiology Research Lab, Army Medical College, Rawalpindi, Pakistan from June 2019 to April 2021 in collaboration with the National Institute of Health (NIH) Islamabad, Pakistan.

Methods: Sixty adult SD rats were divided into three equal groups. Rats were fed on a standard diet as per NIH protocols. After 2 weeks type 2 Diabetes mellitus (T2DM) was induced in groups 2 and 3 by injecting low dose 35mg of streptozotocin (STZ) in the abdomen intraperitoneally. T2DM was successfully developed and confirmed by measuring glucose levels through a glucometer. Group 3 was injected with Alpha Lipoic acid 30mg/kg/day at the lower abdomen for the next two weeks. After 04 weeks, soleus muscles were dissected. The animal data acquisition unit (iWorx) was used for assessing the contractile functions of soleus.

Results: Alpha Lipoic acid group showed improvement in muscle mass, muscle tension strength, and recovery from fatigue after applying fatigue protocol as compared to group 2.

Conclusion: Alpha Lipoic acid supplementation improves contractile force and delays fatigue in the soleus muscles of diabetic rats.

Keywords: *Alpha Lipoic Acid, Blood Glucose, Diabetes Mellitus, Sprague Dawley Rats, Streptozotocin.*

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Introduction

Type 2 Diabetes mellitus (T2DM) is a global epidemic affecting nearly 462 million people worldwide and more than 11 million people in Pakistan.[1] The main pathophysiology of T2DM is peripheral insulin resistance without effective compensation from β-cell—leading to high blood glucose levels and increased lipid metabolism.[2] Skeletal Muscle(SM), one of the largest organ of the body, accounts for 40-50% of the body weight and is composed of two distinct fiber categories: type I slow oxidative and type II fast glycolytic. SM is the most important site for insulin-dependent glucose uptake.[3]

T2DM-associated myopathies are a significant complication affecting our daily physical activities and metabolic well-being.[4] Formation of huge amounts of free radicals which exhaust all the antioxidants in the body, leading to oxidative stress

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(OS) significantly damages the contractile proteins, myocellular lipids, and cellular DNA leading to decreased muscle mass (Atrophy) and impaired contractile functions.[5,6] Furthermore, SM for energy production utilizes plasma glucose and stored muscle glycogen. Oxidative stress in T2DM causes cytotoxic damage to different proteins in mitochondria resulting in mitochondrial dysfunction, with defective oxidative phosphorylation, there is decreased glycogen synthesis and decreased glucose oxidation which further affect muscle fibers' contractile functions by decreasing its oxidative capacity.[7]

Alpha-Lipoic acid ALA is a naturally occurring substance that plays a critical role in mitochondrial dehydrogenases and ATP production.[8] ALA and its important counterpart Dihydrolipoic acid is a powerful and potent antioxidant.[9] ALA has direct effects on skeletal muscles by stimulating glucose uptake through redistribution of insulin-mediated GLUT4 transporters in skeletal muscles and increases insulin sensitivity by breaking down accumulated fatty acid and lipids in skeletal muscle through oxidation by activating AMP-activated protein kinase (AMPK).[10]

Many international and national studies have proven that ALA corrects metabolic derangements and oxidative stress in T2DM however research regarding its effects on contractile functions and skeletal muscle mass is limited. The prime focus of this study was to determine the effect of ALA on slow skeletal muscle (soleus) mass and contractile functions in Streptozotocin (STZ) induced T2DM male SD rats.

Methods

This quasi-experimental study was carried out at the Physiology Research Lab, Army Medical College, Rawalpindi, Pakistan from June 2019 to April 2021 in collaboration with the National Institute of Health (NIH) Islamabad, Pakistan after formal approval from the Ethical Review Committee of the college held on 13th May 2019 vide letter no: 568/Trg. 60 SD male rats, 7-8 weeks old and 235-245gm weight were randomly divided into three equal groups excluding any prior muscle disease and metabolic disease by measuring serum Creatine Phospho-kinase (CPK) levels and glucose levels through glucometer respectively. Rats were fed on a diet prepared as per NIH protocols (Table-1). Rats were kept in steel cages with proper light and dark exposure of 12:12 cycles and optimum temperature maintained at 22-24 ºC in a room with optimum ventilation.

Table-1: Rat's feed

Group 1 (Control)		Group 2 and 3 (Diabetic & ALA)	
Nutrients	**%/100gm**	**Nutrients**	**%/100gm**
Carbohydrates	50%	Carbohydrates	40%
Proteins	20%	Proteins	10%
Fats	30%	Fats	50%
	Composition		
Cellulose	275	NPD	385
Dried milk	285	Casein	315
Mollasen	5	Lard	265
Salt	15	Cholesterol	30
sucrose	50	Vitamin /mineral	70
raw meat	150	L-cystine	3
Vitamins	10	Yeast mixture	05
Wheat brawn	200	Sodium chloride	20

T2DM was induced in groups 2 and 3 by injecting low and single-dose Streptozotocin (40 mg/kg body weight) intraperitoneally after two weeks of diet rich in calories and fat. On the 21st day, the development of T2DM in diabetic and ALA groups was confirmed (plasma glucose level >16.65 mmol/l) by measuring the plasma glucose of all rats by tail vein sampling.

ALA (Thioctacid 600, AstaMedica, Germany) was injected intra-peritoneally (25 mg/kg/day) for two weeks to the ALA group, while normal saline was administered to the other two groups. On the 28th day, rats were euthanized by a high dose of Ether. Soleus muscle was dissected out intact and weight was calculated using a weighing machine and immediately placed in an organ bath system of iWorx (model AHK/214) containing 25ml Krebs-Ringer bicarbonate buffer solution and supplied with 94% O_2 and 6% CO_2 continuously at a fixed temperature of 28-30˚C.11 Force transducer of iWorx Lab-scribe v4 was attached to the tendons for muscle stimulation with stimulation frequencies 10-120 Hz per second and 10 volts for measuring maximum force and peak twitch tension (PTT), time to 50% of the PTT. Peak tetanic force (PTF) and recovery/recouping from fatigue was determined by stimulating muscle with ideal frequency for one minute with rest of 5 seconds in between The mean with standard deviation was calculated using SPSS 22. ANOVA was applied to determine the statistically significant differences across the groups and Post hoc test was applied to confirm the differences among the groups. *P*-value ≤0.05 was recorded as significant.

Results

Body weight and plasma glucose were measured at the end of 3rd week, it showed significant increase in group 2 and 3, confirming the successful induction of T2DM in these two groups (Table-2). Soleus muscle weight is shown in Figure.1 shows a significant decrease ($p \leq .05$) in the diabetic group despite having increased body weight. Skeletal muscle parameters like maximum force, time taken to relax to its maximum strength, and recovery from fatigue after applying the protocol are deranged in group 2 as compared to other groups (Table-3) and after applying Post Hoc Tukey's test it confirmed the difference among the groups. (Table-4).

Table-2: Body weight, Plasma glucose levels cross the Groups at days 1 & 21 (Mean ±SD)

Groups	Days	Control	Diabetic	ALA
Body weight (g)	01	248.45 ± 5.04	248.52 ± 5.47	248.52 ± 5.02
	21	253.63 ± 9.40	271.70 ± 7.35	271.90 ± 8.70
Plasma glucose	01	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

Normal level of plasma glucose≤16.65 mmol/l

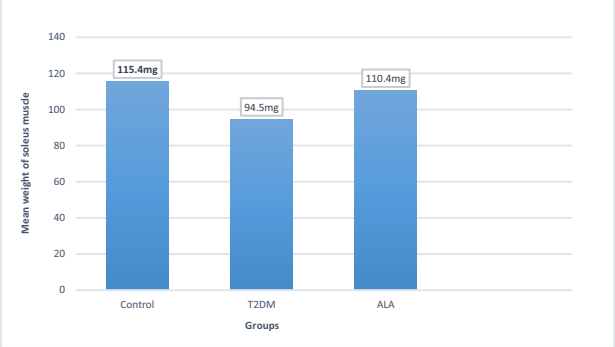

Fig.1: Comparison of isolated soleus muscle weight among study groups

Discussion

Rats and mouse models are extensively used for a better understanding of pathophysiology and disease prognosis of diabetes in many research setups. Rats are preferred over mice in the diabetes model because less time for diabetes induction is taken in rats as compared to mice and they have similar genetic homology with humans in diabetes perspective.12 Srinivasan et al. rat model was used in our study project comprising of the diet with higher quantities of fats for two weeks which causes increased accumulation of myocellular lipids, significant increase in body weights of group 2 ad 3, and thus causing insulin resistance.[13] Low-dose Streptozocin 40mg/kg body weight damages the beta cells of the pancreas causing frank hyperglycemia and confirming the induction of T2DM. 13 The dose of ALA (30 mg/kg bw) for two weeks was used in this study.

Soleus is a slow muscle containing a higher number of oxidative type 1 fibers with abundant mitochondria and a few fast-twitch oxidative glycolytic type 2A fibers.14 There was no significant

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

Contractile properties of soleus muscle	Group I (Control) (Mean±SD)	Group II (Diabetic) (Mean±SD)	Group III (ALA) (Mean±SD)	*P*-value
Twitch				
PTT* (N/g)	1.390 ± 0.021	1.404 ± 0.032	1.398±0.021	0.153
TTP** (ms)	17.83 ± 1.53	21.85 ± 1.23	18.89±1.54	< 0.001
Tetanus				
PTF*** (N/g)	8.65 ± 0.558	8.95 ± 0.481	8.75±.0665	0.126
Recovery from fatigue (PTF after 5 minutes of rest period) (N/g)	8.16 ± 0.609	6.49 ± 0.491	7.98±.498	< 0.001

*TTP** time to peak tension, PTT* peak twitch tension, PTF*** peak tetanic force*

Table-4: Post hoc Tukey's test

Contractile functions isolated soleus muscle	*P*-value		
	Control Rats Vs Diabetic Rats	Control rats Vs ALA rats	Diabetic rats Vs ALA rats
Time To Peak Tension (TTP)	.001	.155	.002
PTF After fatigue protocol	.002	.148	.001

TTP time to peak tension, PTF peak tetanic force

difference in maximum force i.e. PTT among the groups as it depends on available ATPs and calcium ions in sarcoplasm and actin-myosin cross-linkages for a single muscle twitch. Time taken to maximum force TTP is significantly longer in group II, it depends on the release of calcium ions from the sarcoplasmic reticulum (SR) via RyR receptors. Oxidative stress in T2DM has deleterious effects on RyR receptors which delays efficient release of calcium from SR in skeletal muscles.15 Maximum Tetanic force PTF was similar in all groups it depends on calcium ions availability of ATPs and bound cross linkages between actin myosin for stronger generation of tetanic force. Increased lipid accumulation in skeletal muscle cells provides an alternate source of energy for muscle contraction. 16 Repeated Maximum muscle tension and recovery from fatigue in diabetic muscles is less showing its easy fatigue-ability. It depends on a large number of ATPs for sustained tetanic contraction and is provided by already stored muscle glycogen. Diabetic muscles are unable to replenish the energy fuels from the organ bath system because of insulin resistance and oxidative stress also affecting the Glycogen synthase activity causing a significant reduction in glycogen fuel stores. 17 Tamaki, Toru at el. studied the effects of T2DM on soleus muscle and established decreased contractile functions. 18 ALA decreases oxidative stress by reducing ROS and improving insulin sensitivity leading to restoration of glycogen synthase activity which improves glycogen storage and increases glucose reuptake by GLUT4 transporters thus providing a large number of ATPs to produce maximum tension and improved contraction force after fatigue protocol comparable to the controls muscles. Nemati, Samira at el. studied effects of ALA in T2DM rats and established its antioxidant role which supports our study.

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Our study explored the beneficial effects of ALA on contractile functions of slow skeletal muscles and its mass in diabetic rats and can be exogenously used as an adjunct therapy in treating Diabetic myopathies.

Limitations of Study

The mechanism of action of ALA on GLUT4, Ca^{+2} handling, and contractile proteins in Soleus muscles in rats could not be explored in depth due to lack of funding and can be explored by immunohistochemistry in vivo.

Conclusion

T2DM affects the slow muscles peak tetanic tension and causes early fatigability. ALA corrects the oxidative stress and metabolic processes in T2DM which results in better muscle mass, strength, and recovery from fatigue comparable to healthy muscles.

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Authors Contribution

BUK: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing, and proofreading

IY: Idea conception, study designing, data collection, data analysis, results and interpretation, manuscript writing, and proofreading

SA: Idea conception, study designing

FI: Study designing, manuscript writing, and proofreading

AA: Data collection, data analysis, results and interpretation, manuscript writing, and proofreading

IU: Data collection, data analysis, results and interpretation, manuscript writing, and proofreading