TAURINE SUPPLEMENTATION NORMALIZES SKELETAL MUSCLE FUNCTIONS IN TYPE 2 DIABETES RATS

Amina Rasul, Nadia Latif*, Sajid Ali*, Mehvish Ashfaq, Sadia Mubarak***, Irum Rehman******

Al-Razi Medical College, Peshawar Pakistan, *Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **HITEC Institute of Medical Sciences, Taxilla/National University of Medical Sciences (NUMS) Pakistan, ***Islamabad Medical & Dental College, Islamabad Pakistan, ****Margalla Institute of Health Sciences, Rawalpindi Pakistan

ABSTRACT

Objective: To study effect of supplementation of taurine on force frequency relationship and fatigue of rodent skeletal muscles in type 2 diabetes mellitus.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Department of Physiology at Army Medical College Rawalpindi, from Apr 2013 to Jun 2013.

Methodology: Ninety Sprague-Dawley rats were divided randomly in to 3 groups; control (group-1), beta-alanine (group-2), and taurine group (group-3). All rats were fed with taurine free-high fat diet and administered streptozocin to induce type 2 diabetes mellitus. In addition, group-1 was supplemented with 0.02% (w/v) taurine, group-2 with 3% (w/v) beta-alanine, and group-3 with 3% (w/v) taurine in their respective drinking water. At 21st day, plasma glucose and insulin resistance were measured to affirm type-II diabetes mellitus in all groups. At 28th day, contractile functions of extensor digitorum longus muscles at high frequencies were evaluated using i Worx data acquisition unit (AHK/214).

Results: The decline in maximum fused tetanic tension, maximum fused tetanic tension after fatigue protocol, and recovery from fatigue was significantly ameliorated in taurine supplemented diabetic rats.

Conclusion: Taurine supplementation significantly improved the contractile functions of diabetic rodent skeletal muscle at high frequency stimulation.

Keywords: Maximum fused tetanic tension, Maximum fused tetanic tension after fatigue protocol, Taurine.

INTRODUCTION

Type-2 diabetes mellitus (T2DM) is a metabolic disorder that comprises a state of chronic hyperglycemia[1] in which glucose flux through the polyol pathway (PP) increases, with accompanied increase in intramuscular sorbitol[2,3] and decrease in intramuscular Taurine levels[4].

Taurine (Tau), a sulfonic amino acid, is abundantly found in skeletal muscles, with slow oxidative fibres containing approximately twice as much TAU as fast twitch (FT) fibres[5]. Physiological levels of Tau are important for maintaining adequate force responses in FT fibres. Tau enhances the activity of ryanodine receptors, increasing the rate of sarcoplasmic reticulum (SR) Ca^{2+} release. In turn, this directly facilitates Ca^{2+}-dependent excitation-contraction processes, sensitivity of the contractile filaments to Ca^{2+}, and force production. Hence, fibres with a low Tau content would produce significantly less force than those with a high Tau content[6]. Furthermore, Tau forms a conjugate, 5- taurinomethyluridine, with uridine in the mitochondrial transfer RNA which regulates synthesis of mitochondrial-encoded proteins and thereupon, the respiratory chain complexes' activity. Therefore, Tau ensures adequate ATP generation, and prevents excessive mitochondrial superoxide generation[7].

Due to limited endogenous synthesis[7] Tau transporter is identified as a crucial factor for the maintenance of intracellular Tau pool in muscle[8]. Beta-alanine (BA) is an amino acid and because of structural similarity to Tau, it competitively blocks Tau uptake through their common transporter into skeletal muscle, the Tau Transporter (TauT)[9].

Correspondence: Dr Amina Rasul, Asst Prof, Al-Razi Medical College, Peshawar Pakistan (*Email: aminasami@live.com)*
Received: 12 Feb 2019; revised received: 01 Jan 2020; accepted: 10 Feb 2020

The present study was conducted in diabetic Sprague-Dawley rats to evaluate the significance of taurine in rodent skeletal muscle by, down- and up-regulating the Tau content of muscle (with BA and Tau respectively), and examining the effects on skeletal muscle functioning.

METHODOLOGY

This study was performed at department of Physiology, Army Medical College Rawalpindi, in association with National Institute of health Sciences Islamabad, from April to June 2013, after obtaining approval from the Ethical Committee of the aforesaid college. Ninety 60-90 days old, male Sprague-Dawley rats, with a weight of 250 ± 50 grams were selected, after preliminary assessment of plasma glucose and serum creatine phosphokinase levels to eliminate any pre-existing derangement in glucose metabolism and skeletal muscle functions, respectively. Rats were kept in a 2x3 feet steel cages with clean bottles of water fitted over them, in a room well ventilated at 20-22^{o}C on 12 hour alternate cycle of light and dark. Rats were randomly divided in 3 groups-1 (control diabetic), 2 (diabetic BA group) and 3 (diabetic Tau group). All groups were fed on Tau free-high fat diet for 4 weeks. Group-1 was supplemented with 0.02% w/v Tau in drinking water for 4 weeks, group-2 was supplemented with 3% w/v BA, a competitive Tau transport inhibitor, in drinking water for 4 weeks, and group-3 was supplemented with 3% w/v Tau in drinking water for 4 weeks. After 2 weeks, a single intraperitoneal streptozotocin (35mg/kg) injection was administered in the lower-right quadrant of the abdomen of all rats. At the conclusion of 3rd week development of T2DM was established by using cut off value of plasma glucose level >200 mg/dl[10], and HOMA-IR value of >3.9[11] signified the presence of insulin resistance. Rats were continued on the same diet and supplementation for another week. At the end of 4 weeks, rats were euthanized by ether anesthesia overdose. Extensor digitorum longus muscles (EDL) were removed intact and mounted in organ bath system (Harward Apparatus) containing Krebs-Ringer bicarbonate buffer bubbled with 95% O_2 and 5% CO_2 at 30°C. The distal tendons of EDL were fixed to a support and proximal tendons were tied to a force transducer (FT-100) connected to iWorx advanced animal/ human Physiology data acquisition unit; AHK/ 214 (Harvard, USA). Muscles were stimulated supramaximally (5 volts) by electrodes directly placed on the muscle[12]. Optimum length of each muscle was determined by single twitch (1 Hz) electrical stimulation, and maintained for all subsequent stimulations. The force-frequency relationship was determined by stimulation of the EDL muscle for 1 second at frequencies of 10, 30, 50, 70, 90 and 110 Hz with a 3 minutes' rest between each. The maximum fused tetanic tension (MFTT) was recorded and the optimum frequency producing MFTT was also noted. The fatigue characteristics of the muscles were assessed by repeated stimulation with optimum frequency for 1 second, followed by 5 seconds' rest, for a duration of 5 minutes. The muscle function at the end of the fatigue protocol was noted and termed as maximum fused tetanic tension after fatigue protocol (TTFP). And the tetanic tension recorded 5 minutes post fatigue protocol, was termed as recovery from fatigue (RF)[12].

All measured forces were normalized to muscle mass and expressed as Newton per gram (N/g) wet muscle mass. IBM-SPSS v 21 was used to calculate mean with standard deviation of all variables. ANOVA was applied to determine significance among the groups. Post-hoc Tukey test was applied for inter- group comparison.

RESULTS

At the end of 3rd week development of T2DM was established (table-I). When Post hoc Tukey's HSD was applied, a significant improvement in muscle function was observed in Tau treated diabetic rats as compared to diabetic controls and diabetic BA treated rats, whereas insignificant differences were found between control and BA groups (table-II).

DISCUSSION

In the present study, high fat diet-low dose streptozotocin-injected diabetic model was used.

This was preferred because the metabolic profile and natural history optimally resembled that of human T2DM[13].

Rat EDL essentially contains only FT (94%) muscle fibers[6]. In marked contrast to soleus, diabetic EDL muscles exhibited decrement in force production. The results for EDL contractile studies were consistent with the finding of disturbed SR Ca^{2+} handling in fast fibres, driven by the hyperglycemia-induced PP, as the key enzyme aldose reductase is present in skeletal muscle[14]. Enhanced activity of PP led to a reduction in Tau levels and ATP synthesis, preferentially in fast than slow fibers. Therefore, EDL was chosen for the study, as it was expected to benefit from the putative effects of Tau.

In order to elucidate the functional role of intracellular Tau in improving contractile functions of EDL in T2DM, 3% BA, a structural analogue of Tau, was administered to the second study group, which has been documented to reduce Tau content about 50% in all the muscles[15]. Oral administration of a 3% BA solution in the drinking water have been proved to be more convenient, less toxic, and at the same time as effective in increasing urinary Tau excretion in rats, as parenteral administration. 3% Tau was given to the third study group to equal the amount of BA recorded to produce maximal Tau depletion[15]. 3% Tau is documented to significantly increase Tau levels in all muscles except for soleus. Furthermore, diabetic controls were supplemented with 0.02% (w/v) Tau in drinking water, which equals the Tau concentration in standard laboratory rat chow[15].

Besides the effects of different muscle fibre types, the major determinants of tetanic tension are the number of myosin heads interacting with the actin filaments and the availability of Ca^{2+} in the sarcoplasm at any given point in time[16]. Because Ca^{2+} released from the SR instigates the force producing cross bridge cycling, low sarcoplasmic Ca^{2+} due to impaired SR Ca^{2+} release, would make a substantial contribution to the decline in the force output by reducing the capacity of cross bridges to form strong bonds[17]. The 21% decline in tetanic tension seen in EDL of diabetic rats was related to excessive PP activity, because it was offset by aldose reductase inhibitor treatment alone, but was further reduced to 55% of normal with insulin treatment alone. The authors proposed that insulin facilitated the entry of glucose into EDL muscle, producing an overall increase in PP flux. In turn, the increased PP

Table-I: Body weight, plasma glucose, and HOMA-IR in three groups of rats at 21st day.

Parameters	Group-1 (n=30)	Group-2 (n=30)	Group-3 (n=30)
Body weight (g)	308.00 ± 5.18	308.00 ± 6.37	303.67 ± 6.00
Plasma glucose (mg/dl)	254.63 ± 5.18	254.30 ± 5.15	250.27 ± 6.42
HOMA-IR	7.88 ± 1.31	7.91 ± 1.53	5.33 ± 1.21

All values expressed as Mean ± SD, HOMA-IR = Homeostasis model of assessment - insulin resistance

Table-II: Comparison of maximum fused tetanic tension, maximum fused tetanic tension after fatigue protocol, and tetanic tension after 5 minutes of rest period following fatigue protocol among diabetic control, diabetic beta-alanine group, and diabetic taurine group by Post Hoc Tukeys Test at end of the study.

Parameters	Group-1 (n=30)	Group-2 (n=30)	Group-3 (n=30)
MFTT (N/g)			
Mean ± SD	3.932 ± 0.026	3.937 ± 0.022	3.954 ± 0.012
p-value	I & II 0.439	II & III 0.003	I & III <0.001
TTFP (N/g)			
Mean ± SD	1.774 ± 0.066	1.785 ± 0.038	1.814 ± 0.018
p-value	I & II 0.173	II & III <0.001	I & III <0.001
RF (N/g)			
Mean ± SD	3.898± 0.091	3.907 ± 0.088	3.954 ± 0.030
p-value	I & II 0.243	II & III <0.001	I & III <0.001

activity disrupted Ca^{2+} handling and SR morphology. Atrophy of fast muscles was considered a secondary factor[14]. Hence, fibres with a low Tau content would produce significantly less force than those with a high Tau content[6].

In our study, MFTT was decreased in the EDL of the diabetic control rats. Tau supplemented rats exhibited significant increase in force production when compared to controls and BA supplemented rats. Hamilton *et al* decreased EDL Tau levels to <40% of controls by inhibiting the muscle TauT with guanidinoethane sulphate treatment. This was accompanied by significant (p<0.05) reduction in the force output of muscles at stimulation frequencies from 50 to 100 Hz, as compared to controls. The author attributed the force deficit to the Tau depletion[18]. Although we did not measure the muscle Tau content, but 3% BA supplementation for a period of 4 weeks had been documented to cause a 50% reduction in muscle Tau content as compared to controls in the rodent EDL (7.32 ± 0.32 µmoles/g wet tissue weight in BA EDL vs 14.11 ± 0.95 µmoles/g wet tissue weight in control EDL[15]. This, combined with further Tau loss through increased PP activity in diabetes in the BA supplemented rats in the present study, resulted in decreased SR Ca^{2+} release per action potential and hence the decline in maximum force output. For the Tau supplemented group, the dose (3%) and duration (4 weeks) of Tau was adequate to optimally counter the Tau deficit that was produced in BA rats (19.92 ± 0.46 µmoles/g wet tissue weight in Tau EDL vs 7.32 ± 0.32 µmoles/g wet tissue weight in BA EDL)[15] and as a result, improve SR Ca^{2+} release and muscle force output. When skinned muscle fibers deficient of most Tau were bathed in physiological doses of Tau, both SR Ca^{2+} release and the sensitivity of contractile filaments to Ca^{2+} were augmented[6]. However, Tallis *et al* reported no effect of physiological concentration of Tau on force output of isolated mouse soleus muscle[19]. It could be because in rat skeletal muscle, Tau concentration is higher in slow twitch soleus muscle (33 µmol/g wet weight) as compared to FT EDL (17 µmol/g wet weight) and therefore, physiological dose of Tau did not further alter the soleus Ca^{2+} response to electrical stimulation[19].

In the present study, the diabetic rats exhibited decreased EDL contraction force after fatigue protocol. The Tau supplemented rats exhibited better force production and resistance to fatigue, as compared to control and BA supplemented rats.

T2DM reduces glycogen content of skeletal muscle and produces mitochondrial dysfunction leading to impaired ATP production. With prolonged exercise, the energy demand of skeletal muscle increases tremendously, but because of reduced glycogen content ATP is exhausted at significantly greater rate[12]. Moreover high flux through PP also reduces net ATP synthesis[14] and induces alterations in the redox state of the muscle, increasing oxidative stress[2]. Resultantly, there is greater decline in muscle performance during a continuous activity.

In the current study, fatigue resistance (TTFP) in the EDL muscle was greater in the Tau group. This could be because Tau not only enhances the glucose uptake in skeletal muscles[20], but also increases the muscle glycogen content in the diabetic rat model[21]. Harada *et al* and Ishikura *et al* also showed similar results. In their study, Tau was reported to be indirectly involved in the process of hepatic gluconeogenesis in FT gastrocnemius muscle in normal as well as Zucker diabetic fatty rats, after 2 weeks of oral Tau (3% solution in drinking water) supplementation. This was accompanied with significantly (p<0.05) increased running time to exhaustion, as compared to the untreated control rats[21,2]. In a different approach, TauT-knockout (TauTKO) mice containing severely compromised skeletal muscle Tau levels, exhibited reduction in treadmill running speed and endurance time as compared to control mice. Moreover, blood lactate levels were >3 fold increased, and impaired flux through the respiratory chain (which increased the NADH/NAD+ ratio) indicating ineffective ATP generation, was reflected by diminished ATP levels of gastrocne-

mius during treadmill running. Because these mice exhibited augmented energy metabolism despite having compromised exercise ability, the authors justified that at greater exercise loads the increased call for energy production exceeded muscle capacity to produce ATP further impairing muscle performance[8].

The RF also showed statistically significant (p<0.001) difference between rats. Tau supplementation was associated with faster recovery from fatigue as compared to the BA treated group. In contrast to EDL muscle of the diabetic control rats, the Tau treated diabetic rats, on account of improved insulin sensitivity, may have been better able to take up glucose from the surrounding buffer medium and utilize it, thus replenishing ATP stores. Collectively, these actions of Tau had a positive effect on energy metabolism during recovery from fatigue induced by high frequency stimulation[23] that contributed to the rapid recovery of mechanical function observed. Ishikura *et al* demonstrated that Tau treatment prevented the exercise-induced decrease in muscle Tau concentration in the FT muscle and reduced muscle damage that occurred during exercise[22].

However, no significant difference was found in all contractile parameters between control and BA (Tau downregulated) rats. This could probably be because of the ergogenic effect of BA not discussed here, and also because the controls were supplemented with 0.02% (w/v) taurine in the drinking water which equals the Tau content of standard laboratory rat chow[14] and hence they represented control diabetic rats with no intervention and in which Tau levels were not much decreased during the course of diabetes, to significantly decrease muscle function as compared to BA rats.

CONCLUSION

Tau treatment improved the skeletal muscle contractile functions in T2DM rat and it can work as an adjunct therapeutic agent in treatment of T2DM.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(Suppl-1): S81-90.
2. Sanchez OA, Walseth TF, Snow LM, Serfass RC, Thompson LV. Skeletal muscle sorbitol levels in diabetic rats with and without insulin therapy and endurance exercise training. Exp Diabetes Res 2009; 2009: 737686.
3. Yan LJ. Redox imbalance stress in diabetes mellitus: Role of the polyol pathway. Animal Model Exp Med 2018; 1(1): 7-13.
4. De Luca G, Calpona PR, Caponetti A, Romano G, Di Benedetto A, Cucinotta D, et al. Taurine and osmoregulation: platelet taurine content, uptake, and release in type 2 diabetic patients. Metabolism 2001; 50(1): 60-64.
5. Dutka TL, Lamboley CR, Murphy RM, Lamb GD. Acute effects of taurine on sarcoplasmic reticulum Ca^{2+} accumulation and contractility in human type I and type II skeletal muscle fibers. J Appl Physiol 2014; 117(7): 797-805.
6. Bakker AJ, Berg HM. Effect of taurine on sarcoplasmic reticulum function and force in skinned fast-twitch skeletal muscle fibres of the rat. J Physiol 2002; 538(Pt1): 185-94.
7. Schaffer SW, Ito T, Azuma J. Clinical significance of taurine. Amino Acids 2014; 46(1): 1-5.
8. Ito T, Yoshikawa N, Schaffer SW, Azuma J. Tissue taurine depletion alters metabolic response to exercise and reduces running capacity in mice. J Amino Acids 2014; 2014: 964680.
9. Trexler ET, Smith-Ryan AE, Stout JR, Hoffman JR, Wilborn CD, Sale C, et al. International society of sports nutrition position stand: Beta-Alanine. J Int Soc Sports Nutr 2015; 12(1): 30-34.
10. Husni A, Purwanti D, Ustadi U. Blood glucose level and lipid profile of streptozotocin-induced diabetes rats treated with sodium alginate from sargassum crassifolium. J Biol Sci 2016; 16(3): 58-64.
11. Antunes LC, Elkfury JL, Jornada MN, Foletto KC, Bertoluci MC. Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Arch Endocrinol Metab 2016; 60(2): 138-42.
12. Aleem SB, Hussain MM, Farooq Y. Serum levo-carnitine and skeletal muscle functions in type 2 diabetes mellitus in rodents. J Coll Physicians Surg Pak 2013; 23(2): 132-36.
13. Holmes A, Coppey LJ, Davidson EP, Yorek MA. Rat models of diet-induced obesity and high fat/low dose streptozotocin type 2 diabetes: Effect of reversal of high fat diet compared to treatment with enalapril or menhaden oil on glucose utilization and neuropathic endpoints. J Diabetes Res 2015; 2015: 307285.
14. Cameron NE, Cotter MA, Robertson S. Changes in skeletal muscle contractile properties in streptozocin-induced diabetic rats and role of polyol pathway and hypoinsulinemia. Diabetes 1990; 39(4): 460-65.
15. Dawson R, Biasetti M, Messina S, Dominy J. The cytoprotective role of taurine in exercise-induced muscle injury. Amino Acids 2002; 22(4): 309-24.
16. Huang L, Chen L, Qiu Y, Li S. Abnormalities in the fibre composition and contractility in diabetic skeletal muscles. Int J Clin Exp Med 2018; 11(2): 753-63.
17. Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: general understanding and treatment. Exp Mol Med 2017; 49(10): e384.
18. Hamilton EJ, Berg HM, Easton CJ, Bakker AJ. The effect of taurine depletion on the contractile properties and fatigue in

fast-twitch skeletal muscle of the mouse. Amino Acids 2006; 31(3): 273-78.
19. Tallis J, Higgins MF, Cox VM, Duncan MJ, James RS. Does a physiological concentration of taurine increase acute muscle power output, time to fatigue, and recovery in isolated mouse soleus (slow) muscle with or without the presence of caffeine? Can J Physiol Pharmacol 2014; 92(1): 42-49.
20. Carneiro EM, Latorraca MQ, Araujo E, Beltra M, Oliveras MJ. Taurine supplementation modulates glucose homeostasis and islet function. J Nutr Biochem 2009; 20(7): 503-11.
21. Harada N, Ninomiya C, Osako Y, Morishima M, Mawatari K, Takahashi A, et al. Taurine alters respiratory gas exchange and nutrient metabolism in type 2 diabetic rats. Obes Res 2004; 12(7): 1077-84.
22. Ishikura K, Miyazaki T, Ra SG, Endo S, Nakamura Y, Matsuzaka T, et al. Effect of taurine supplementation on the alterations in amino acid content in skeletal muscle with exercise in rat. J Sports Sci Med 2011; 10(2): 306-14.
23. Takahashi Y, Urushibata E, Hatta H. Higher voluntary wheel running activity following endurance exercise due to oral taurine administration in mice. J Phys Fitness Sports Med 2013; 2(3): 373-79.

.....