ORIGINAL ARTICLE

EFFECT OF LEVOCARNITINE ON ENDURANCE CAPACITY IN TYPE-2 DIABETIC RATS

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Background: Carnitine is an essential cofactor for the enzymes transporting long chain fatty acids across mitochondrial membranes for beta oxidation and also modulates the intra-mitochondrial acylCoA/CoA ratio. This study was conducted to determine the effect of levo-carnitine on endurance capacity, skeletal muscle fatigue characteristics and glycogen stores in diabetic rats. **Methods:** This laboratory based experimental study was conducted in department of Physiology, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from June 2009 to July 2010. The study was carried on 60 healthy male Sprague-Dawley rats. Serum creatine phosphorkinase (CPK) levels were measured to exclude skeletal muscle disorder**.** Rats were fed high fat diet (2 weeks) followed by intra-peritoneal injection of streptozocin (35 mg/kg). On 21st day, after confirmation of type 2 diabetes by measuring plasma glucose and TG/HDL ratio, rats were divided into 2 equal groups; group I (Diabetic) and group II (Carnitine). Group II was administered l-carnitine (200mg/kg) for 6 days. Both groups were further subdivided into 2 equal groups- a (swim group) and b (non-swim group). At end of 4th week, the rats of swim group were subjected to swimming test. The extensor digitorum muscle (EDL) of rats of non-swim group was dissected for evaluation of skeletal muscle fatigue characteristics. The glycogen content of EDL muscle and serum free carnitine (FC) levels of all groups were measured. **Results:** Carnitine treated rats exhibited improvement in swim time as well as skeletal muscle glycogen stores (p<0.001). Significant improvement was also observed in skeletal muscle fatigue characteristics (p<0.05). Serum free carnitine levels were also significantly raised in carnitine groups; the swim groups showed a lower FC levels as compared to their respective non-swim groups (p<0.001). **Conclusion:** Levo-carnitine increases the glycogen stores and improves the skeletal muscle fatigue characteristics, leading to improvement in endurance capacity in type 2 diabetic rats.

Keywords: type 2 diabetes, levo-carnitine, endurance, skeletal muscle, muscle glycogen store

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INTRODUCTION

Skeletal muscle takes up major part of postprandial glucose, and hence it is the main site of insulin resistance, which is a major characteristic of type 2 diabetes mellitus (T2DM).[1] T2DM is known to have decreased insulin-dependent pyruvate dehydrogenase (PDH) activity resulting in decreased glucose oxidation[2] and reduced muscle glycogen content especially in type II muscle fibers[3]. During exercise, skeletal muscles utilize muscle glycogen and plasma glucose by oxidation in order to supply energy for contraction.[4] Thus low skeletal muscle glycogen stores and impaired glucose metabolism are likely to cause easy muscle fatigue in diabetic patients during exercise.

Carnitine (L-3-hydroxy trimethyl aminobutanoate), a naturally occurring compound synthesized in mammals from the essential amino acids lysine and methionine,[5] is an essential cofactor for the enzymes transporting long chain fatty acids across mitochondrial membranes for beta oxidation.[6] Carnitine also modulates the intra-mitochondrial acylCoA/CoA ratio.[7] More than 95% of total body carnitine is present in the skeletal muscle.[8] L-carnitine has been documented to activate pyruvate dehydrogenase complex, increase insulin sensitivity, resulting in improved glucose oxidation.[2]

Previous studies have reported a favourable effect of levo-carnitine on endurance capacity in healthy rats.[9,10] The present study was designed to investigate the effect of this drug supplement on skeletal muscle endurance capacity in type 2 diabetes by measuring the skeletal muscle fatigue characteristics as well as measurement of skeletal muscle glycogen content and serum free carnitine levels.

MATERIAL AND METHODS

This laboratory based experimental study was conducted in department of Physiology, Army Medical College, Rawalpindi, in collaboration with NIH, Islamabad, from June 2009 to July 2010.

Sixty healthy male Sprague Dawley rats were induced type 2 diabetes by administering single dose of streptozotocine (35mg/kg, intraperitoneally)[11] after feeding them 2 weeks high fat diet.[12] A week later, diabetes was confirmed by measuring plasma glucose (>200 mg/dl)[13] and insulin resistance (TG: HDL ratio >1.8)[14] by drawing tail vein blood[15]. Rats were then

subjected to 24 hours fast in order to decrease glycogen content.[16] Rats were then divided into two equal groups, group I (diabetic group) and group II (Carnitine group).

Group II rats were administered levo-carnitine for 6 days, at a dose of 200 mg/kg intra-peritoneally. Each group was then subdivided into two equal groups- a (swim group) and b (non swim group). The rats of first sub-groups of each group underwent swimming until exhaustion in order to evaluate the endurance capacity. Swimming was carried out in a glass water tank (80×50×50 cm), that was filled up to 40 cm water[10], temperature of water was set at 35 °C in order to elicit maximal response in prolonged swimming. The uncoordinated movements and remaining under water for 7 seconds without swimming at the surface was taken as the exhaustion criteria of the rats.[17]

The rats of non-swim subgroups were evaluated for contractile properties in which the muscle fatigue characteristics (maximum fused tetanic tension, maximum fused tetanic tension after fatigue protocol and tetanic tension after 5 minutes rest period following fatigue protocol) were assessed. After anaesthesia, the EDL muscle was dissected and mounted in 25 ml chamber of organ bath system containing Krebs–Ringer bicarbonate buffer (pH=7.3, temperature=30 °C). The solution was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 .[18] The four distal EDL tendons were fixed to a support while proximal tendon was tied to the force transducer (FT-100)[17] connected to iWorx advanced animal/human Physiology data acquisition unit (AHK/214). Contractions were evoked by supramaximal stimulation (5 volts)[19] via platinum electrodes placed directly on the muscle.

The maximum fused tetanic tension was recorded by stimulating the muscle at increasing frequency rate for one second with 3 minutes rest period. The frequency that produced maximum fused tension was termed as the optimum frequency. The muscle was then stimulated with the optimum frequency for 5 minutes with 5 second interval. The muscle response was expressed as a decline in force from the initial maximum fused tetanic tension. A measure of recovery from fatigue was also made by recording the tetanic tension after a 5 minutes rest period following the tetanic contractions. All measured forces were normalized to muscle mass and expressed as Newton per gram (N/g) wet muscle mass.[18]

The EDL muscle of all rats was assessed for glycogen content (in case of swim groups, the rats were immediately dissected after swimming), using an anthrone based method.[20] Serum free carnitine levels of all the rats were measured from terminal intra-cardiac blood (in case of swim groups, blood sample was drawn immediately after swimming), using l-carnitine assay kit.

Data was analysed using SPSS-17. Mean and standard deviation were calculated for all values. Statistical significance of difference between the subgroups was determined by applying independent samples *t*-test. ($p \leq 0.05$ was considered significant).

RESULTS

The swim time (endurance capacity) was increased in carnitine group IIa (34.5±2.37 minutes) as compared to the diabetic group Ia (22.20±3.79 minutes). This can be correlated with the improvement in glycogen content observed in carnitine group IIa (124.2±17.78 mg/100 gm muscle) as compared to diabetic group Ia (82.55±10.30 mg/100 gm muscle). The muscle glycogen content was found significantly reduced after the swim test in group Ia (19.9±6.47 mg per 100 gm muscle) and group IIa (18.8±4.29 mg per 100 gm muscle) (Table-1).

The serum free carnitine levels showed a significant rise in carnitine swims and carnitine non-swim group as compared to the respective diabetic swim non swim group. However, the carnitine levels of the swim groups had comparatively lower free carnitine levels, as compared to their respective non swim groups ($p<0.001$) (Table-1,2).

Significant improvement was observed in maximum fused tetanic tension, maximum fused tetanic tension after fatigue protocol and recovery from fatigue after 5 minutes of rest period when diabetic non swim group was compared with carnitine non swim ($p<0.05$) (Table-3).

Table-1: Swim time, serum free carnitine levels and EDL muscle glycogen content of all groups

Variables	Group Ia (Diabetic swim) n=15	Group Ib (Diabetic non-swim) n=15	Group II a (Carnitine swim) n=15	Group II b (Carnitine non-swim) n=15
Swim time (min)	22.20±3.79	-	34.5±2.37	-
Carnitine levels (nmol/µl)	0.063±0.028	0.109±0.014	0.176±0.035	0.312±0.158
Glycogen content (mg per 100gm)	19.90±6.47	82.55±10.30	18.80±4.29	124.2±17.78

All values are expressed as Mean±SD

Table-2: Comparison of swim time, serum free carnitine levels and EDL muscle glycogen content between all groups

Variables	Ia v/s Ib (*p* value)	IIa v/s IIb (*p* value)	Ia v/s IIa (*p* value)	Ib v/s IIb (p value)
Carnitine levels (nmol/µl)	<0.001	<0.001	<0.001	<0.001
Swim time (min)	-	-	<0.001	-
Muscle glycogen content (mg per 100gm)	<0.001	<0.001	0.641	<0.001

Table-3: Comparison of maximum fused tetanic tension, maximum fused titanic tension after fatigue protocol and tetanic tension after 5 minutes of rest period following fatigue protocol between non swim groups

Variables	Group Ib (n=15)	Group IIb (n=15)	*p*-value
Maximum fused tetanic tension (N/g)	4.088±0.062	4.5340±0.325	<0.001
Maximm fused tetanic tension after fatigue protocol (N/g)	1.722±0.050	2.306±0.416	0.002
Tetanic tension after 5 minutes of rest period following fatigue protocol (N/g)	3.218±0.050	3.977±0.260	<0.001

All values are expressed as Mean±SD

DISCUSSION

Our observations indicated that levo-carnitine improved the endurance capacity in T2DM. The swimming test was selected because exhaustion is more clearly observed in rodents during swimming than in running. Swimming sessions were also carried for few days prior to study to avoid data scatter. In current study, administration of levo-carnitine led to improvement in swim time. Previous study had reported a longer swim time when a similar dose of carnitine administered to healthy mice for a similar duration.[10]

The improvement in endurance capacity in current study can be correlated with improvement in the muscle glycogen content in carnitine non swim group as compared to diabetic non swim group. During intense activity, there is a high glycolytic rate leading to utilization of glycogen reserves in the contracting muscle especially in the fast-twitch glycolytic muscle fibres.[21,22] The link between elevated glycogen store and improved exercise performance, as evident in various studies,[4,23] has also been revalidated in current study. Therefore, one of the possible causes for improvement in endurance capacity in the carnitine treated rats was the increase in muscle glycogen as energy substrate.

Study of skeletal muscle fatigue characteristics was done on fast twitch (type II) glycogen rich EDL muscle. The type II muscle fibres particularly have been found to be insulin-resistant.[24] The maximum fused tension obtained by increasing stimuli showed an improvement in carnitine treated rats. A significant improvement in force was observed in the carnitine non swim group as compared to the diabetic non swim group, when the EDL muscle was stimulated with repeated tetanic stimulations, at 5seconds interval for 5 minutes.

In study by Bruton *et al*., a rapid reduction in force as well as myoplasmic Ca^{2+} was recorded in EDL muscle of genetically obese mice, when the muscle was stimulated by 50 repeated 70Hz stimuli at 2 seconds interval. This was attributed to lower glycogen content and abnormal Ca^{2+} handling ability in those mice as compared to normal mice.[25] Increased carnitine concentration had been documented to delay muscle fatigue in rats in vitro muscle systems.[9] Levo-carnitine has been documented to normalize the PDH activity, stimulate oxidative utilization of glucose and reduce lactate levels in plasma and skeletal muscles.[2,26] All these changes probably led to the increased glycogen content of skeletal muscle as observed in present study and resulted in improvement of the peak tetanic force production.

In the current study, the recording of force on tetanic stimulation following 5 minutes of rest has revealed an improvement in carnitine group as compared to diabetic group. The muscles of the carnitine group were able to take up adequate glucose from the buffer medium in which they were placed, thus resulting in better replenishment of ATP stores. [27]

The present study also showed decrease in serum free carnitine levels (FC) in both exercise and non exercise groups of carnitine group as compared to the respective diabetic groups. Studies have documented low FC and increased esterified carnitine levels in diabetic patients.[28] The swim groups depicted reduced FC levels compared to the respective non swim group. During high intensity exercise, a higher rate of acetyl CoA is produced as compared to its utilization by tricarboxylic acid cycle resulting in decline in free carnitine levels.[29] Study by van Loon and colleagues have also reported a decrease in free carnitine levels in parallel with increase in exercise intensity.[30]

Thus, the present study showed that levo-carnitine supplementation in the carnitine group enhanced the endurance capacity. This was further supported by the improvement in skeletal muscle fatigue characteristics and presence of higher muscle glycogen content after levo-carnitine administration.

CONCLUSION

Levo-carnitine increases the skeletal muscle glycogen stores in T2DM which leads to improvement in skeletal muscle fatigue characteristics, thereby leading to improved endurance capacity.

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