Levo-carnitine supplementation normalizes skeletal muscle functions in type 2 diabetic rats

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Abstract
Levo-carnitine is known to improve skeletal muscle contractile functions in healthy humans and rats. It has also been claimed to correct metabolic derangements in type 2 diabetes mellitus (T2DM). This randomized control trial was designed to determine the effect of *levo*-carnitine on isometric contraction, force frequency relationship and fatigue of skeletal muscles along with oxidative stress in T2DM. Sprague-dawley rats (n=90) were randomly divided in three equal groups. Control group remained healthy while T2DM was induced in the diabetic and carnitine groups. Then, carnitine group was administered *levo*-carnitine 200mg/kg/day intraperitonealy for 6 days. At 28th day, extensor digitorum longus (EDL) muscles were removed and their functions were assessed using iWorx data acquisition unit (AHK/214). Blood obtained by intra-cardiac sampling at 28th day was used for estimation of serum malondialdehyde (MDA) levels. Regarding muscle parameters, no significant difference was found in maximum isometric twitch tension, time-to-peak twitch tension and time-to-relax to 50% of the peak twitch tension amongst the three groups. However, carnitine group showed significant improvement in maximum fused tetanic tension, maximum fused tetanic tension after fatigue protocol and recovery from fatigue after 5 minutes of rest period when compared with diabetic group. Serum MDA levels were reduced in carnitine group as compared to the diabetic group. These findings suggest that *levo*-carnitine supplementation reduces oxidative stress, improves work capacity and delays fatigue in T2DM.

Keywords: *Levo-carnitine, type 2 diabetes mellitus, skeletal muscle functions, oxidative stress.*

Introduction

Skeletal muscle is a major site of impaired insulin action in noninsulin dependent diabetes mellitus; called type 2 diabetes mellitus (T2DM). It has been established that insulin-stimulated rate of glycogen synthesis in muscles is markedly reduced in T2DM[1]. Statistically significant reduction in muscle glycogen has been observed in type II muscle fibers.[2] Furthermore, in T2DM patients, insulin-dependent pyruvate dehydrogenase (PDH) activity appears to be reduced which is considered to be the cause of reduced insulin-stimulated glucose oxidation in such patients.[3] In T2DM, generation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radical is increased in various tissues including skeletal muscles.[4] These products are cytotoxic and highly reactive, leading to free-radical damage to the mitochondrial proteins and reduction in oxidative capacity of the skeletal muscles.[5] These metabolic derangements are likely to affect skeletal muscle contractile parameters adversely.

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Levo-carnitine, also called *β*-hydroxy-*γ*-trimethylamino-butyrate, is a known transporter of long-chain fatty acids across the inner mitochondrial membrane into the matrix for *β*-oxidation.[6] *Levo*-carnitine supplementation improves skeletal muscle adaptation to strenuous exercise in healthy athletes, delays muscle fatigue and permits improved maintenance of contractile force both in humans and animals.[11] Human studies also demonstrate a better recovery from fatigue in individuals taking *levo*-carnitine supplement and significantly reduced post exercise muscle damage[12] and pain.[13] Studies have shown a significant reduction in the malondialdehyde (MDA) concentration, a marker of the level of ROS-induced lipid peroxidation, in rats receiving *levo*-carnitine. This demonstrates the effect of *levo*-carnitine in protecting the ROS mediated muscle damage produced by the forced muscular exercise.[11,14]

Plasma *levo*-carnitine levels decrease in T2DM patients[7] and its supplementation significantly improves insulin sensitivity, glucose uptake and glucose oxidation by skeletal muscles in T2DM patients.[8] Plasma lactate levels significantly decrease after *l*-carnitine administration in T2DM because of PDH activation.[9] *L*-carnitine also decreases the oxidative stress in T2DM.[10] These findings manifest the ability of *l*-carnitine to normalize metabolic derangements in T2DM.

Keeping in view, the present study was planned in insulin resistant type 2 diabetic Sprague-Dawley rats to determine the effect of *levo*-carnitine on isometric contraction, force frequency relationship and fatigue of skeletal muscles.

Material and Methods

This randomized control trial was carried out in Physiology department, Army Medical College, Rawalpindi, after permission by the Ethical Committee of the said institution. 90 healthy Sprague-Dawley rats, 80 ± 5 days old with average weight of 250 ± 50 grams. Plasma glucose and serum creatine phosphokinase levels were assessed to rule out any derangement in glucose metabolism and skeletal muscle disease. Rats were kept in 2 x 3 feet steel cages with clean water bottles specific to fit over these cages in a well ventilated room and cycles of 12 hour light and 12 hour dark were maintained under a temperature range of 20-22°C. Rats were randomly divided into ‘Control’ ‘Diabetic’ and ‘Carnitine’ groups each having 30 rats. Control rats were fed on normal diet ad libitum while diabetic and carnitine group rats were fed high fat diet for 28 days. The composition of food given to the groups is presented in table 1.

After 2 weeks, Streptozocin (35 mg/kg) was given intraperitonealy in the lower-right quadrant of the abdomen to the diabetic and carnitine groups while the control rats instead received the normal saline.[15] At 21st day, body weight, plasma glucose and triglyceride to high density lipoproteins (TG:HDL) ratio of all rats were measured by tail vein sampling and development of T2DM in diabetic and carnitne groups was confirmed.[16] Afterwards, rats were subjected to an overnight fast of 24 hours.[17]

Serum malondialdehyde levels

Body weight of the rats was measured at 28th day after which they were anaesthetized and blood was obtained by intra-cardiac sampling for estimation of serum malondialdehyde levels by enzymatic caloric method. Standard calibration curve was plotted by plotting corrected absorbance values. The concentration of malondialdehyde was calculated and recorded according to the sample number.Plasma glucose and serum TG: HDL ratio were also estimated.

Skeletal muscle functions

At 28th day, rats were anaesthetized and extensor digitorum longus muscles (EDL) were removed intact by first opening the femoral compartment and cutting the proximal tendon. Skin and the biceps femoris muscle were removed to expose anterior crural compartment. Distal fasciotomy was performed along with the removal of connective tissue and ligaments to dissect out the distal tendons of EDL intact from the foot. The muscles were mounted in 25-ml chamber of organ bath system (Harward Apparatus), containing Krebs–Ringer bicarbonate buffer. The temperature of the organ bath was maintained at 30 ^{0}C with the help of thermostat and it was continuously bubbled with a mixture of 95% O_2 and 5% CO_2.[18] The four distal EDL tendons were tied together by non-absorbable surgical silk and fixed to a support while proximal tendon was tied to the force transducer (FT-100) connected to iWorx advanced animal/human physiology data acquisition unit (AHK/214). Muscles were evoked by supramaximal stimulation (5 volts)[19] via platinum electrodes placed directly on the muscle aligned in a parallel direction to the muscles longitudinal axis.

Measurement of isometric contractions

Single twitch (1 Hz) electrical stimulations, with one minute rest periods in between were used to determine the optimum length of each muscle at which maximum twitch tension was produced. The length of the muscle was adjusted via micromanipulator and the optimum length was maintained for all subsequent stimulations. Maximum isometric twitch tension, time-to-peak twitch tension & time taken to relax to 50% of the peak twitch tension was noted.[18]

The force-frequency relationship

The tension produced in the muscles by stimulation at frequencies of 10, 30, 50, 70, 90 and 110 Hz for 1 second was recorded. Three minutes rest period was given after each stimulation. The maximum fused tetanic tension was calculated and the optimum frequency that produced this tension was noted.[18]

Muscle fatigue

Muscles were stimulated at optimum frequency for 1 second and the recording was termed as the initial maximum fused tension. The muscle was stimulated with the same stimulus with 5 seconds rest period in between, for a total period of 5 minutes. The muscle response was recorded for every subsequent minute from the initial recording, for a total period of 5 minutes and 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 fatigue protocol.[18]
All measured forces were normalized to muscle mass and expressed as Newton per gram (N/g) wet muscle mass.[20] Mean with standard deviation of all variables were calculated and statistical significance of differences across the groups was determined by ANOWA followed by post hoc test (Tukey’s HSD). P value < 0.05 was considered significant.

Results

The initial body weight, plasma glucose and serum creatine phosphokinase levels of the rats were within normal range. Development of T2DM and insulin resistance was confirmed at the end of 3rd week of study (table 2). According to the criteria laid down by Yassin and Mwafy for Sprague-Dawley rats, plasma glucose level of > 200 mg/dl was used as the “cut off value” of hyperglycemia for confirming T2DM.[21] Since insulin resistance cannot be measured directly, therefore TG:HDL ratio was used as surrogate marker. The cut off value of ratio as 1.8 was used to mark insulin resistance.[22] The body weight of each rat was measured at end of 3rd week and was found to be increased in the diabetic and carnitine groups due to high fat diet as compared to the controls.

Table 1: Food composition given to the groups

Control group		Diabetic & carnitine groups	
Ingredients	***Weight (g/kg)***	***Ingredients***	***Weight (g/kg)***
Wheat flour	285	Casein	254
Wheat brawn	285	Cornstarch	169
Salt (common)	5	Sucrose	85
Mollasen	15	Wheat bran	51
Soybean oil (ml/kg)	50	Safflower oil (ml/kg)	339
Fish meat	150	Gelatin	19
Vitamins/ minerals	10	Salt mix	67
Dried skimmed milk	200	Vitamin e acetate (500 iu/g)	0.31
		VITAMIN MIX	13
		DL-Methionine/L-cystine	3

Table 2: Body weight, plasma glucose and TG:HDL in three groups of rats at 21st day of the study

Variables	**Control Group n = 30**	**Diabetic Group n = 30**	**Carnitine Group n = 30**
Body weight (g)	256.63 ± 7.40	269.70 ± 8.35	268.80 ± 7.70
Plasma glucose (mg/dl)	105.20 ± 5.70	416.82 ± 7.36	412.69 ± 7.54
TG : HDL	1.37 ± 0.55	2.17 ± 0.78	2.16 ± 0.92

All values have been expressed as Mean ± SD

Table 3: Body weight, plasma glucose and TG:HDL in three groups of rats 28th day of the study

Variables	**Control Group n = 30**	**Diabetic Group n = 30**	**Carnitine Group n = 30**
Body weight (g)	265.71 ± 7.15	278.70 ± 7.61	269.80 ± 8.34
Plasma glucose (mg/dl)	105.20 ± 5.70	430.69 ± 8.54	154.20 ± 5.70
TG : HDL	1.38 ± 0.38	2.40 ± 0.84	1.56 ± 0.24

All values have been expressed as Mean ± SD

Table 4: Comparison of maximum isometric twitch tension, time to peak twitch tension, time taken to relax from maximum twitch tension to its 50% at 28th day

Variables	**Control Group n = 30**	**Diabetic Group n = 30**	**Carnitine Group n = 30**	**p Value**
Maximum isometric twitch tension (N/g)	0.32 ± 0.04	0.31 ± 0.03	0.31 ± 0.04	0.13
Time to peak twitch tension (ms)	19.6 ± 3.3	21.6 ± 3.9	20.7 ± 3.6	0.79
Time taken to relax from maximum twitch tension to its 50% (ms)	20.5 ± 3.2	21.8 ± 3.5	21.3 ± 3.1	.76

All values have been expressed as Mean ± SD

Plasma glucose, TG: HDL ratio and body weight of each rat was measured at 28th day. The values remained deranged in the diabetic groups while the biochemical parameters showed normalization in the carnitine group and there was minimal increase in the body weight despite feeding high fat diet (table 3).

Serum malondialdehyde levels

Serum malondialdehyde levels estimated at the end of the study were reduced significantly in the diabetic group as compared to the controls and carnitine group (figure 1). Application of Post Hoc Test (Tukey's HSD) showed that all the groups significantly differed from each other.

Skeletal muscle function

There was no statistically significant difference among the groups in maximum isometric twitch tension ($p < 0.13$), time to peak twitch tension ($p < 0.79$) and time taken to relax from maximum twitch tension to its 50% ($p < 0.76$) (table 4).

Statistically significant difference was found among the groups in maximum fused tetanic tension ($p < 0.01$), maximum fused tetanic tension after fatigue protocol ($p < 0.02$) and tetanic tension measured after the period of 5 minutes of rest following the fatigue protocol ($p < 0.02$), when one way ANOVA was applied (table 5). After application of post hoc test (Tukey's HSD) the diabetic group was found significantly different from other groups while control and carnitine groups had insignificant differences (table 6).

Table 5: 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 at 28th day

Variables	Control Group n = 30	Diabetic Group n = 30	Carnitine Group n = 30	p Value
Maximum fused tetanic tension (N/g)	4.000 ± 0.006	3.975 ± 0.061	3.999 ± 0.008	0.014
Maximum fused tetanic tension after fatigue protocol (N/g)	1.813 ± 0.029	1.787 ± 0.054	1.812 ± 0.030	0.020
Tetanic tension after 5 minutes of rest period following fatigue protocol (N/g)	3.968 ± 0.029	3.936 ± 0.074	3.967 ± 0.027	0.021

All values have been expressed as Mean ± SD

Table 6: Comparison of Control, Diabetic and Carnitine groups using Pot Hoc Test (Tukey's HSD)

	'p' value		
Variables	Control vs Diabetic	Control vs Carnitine	Diabetic vs Carnitine
Maximum fused tetanic tension	0.024	0.989	0.035
Maximum fused tetanic tension after fatigue protocol	0.035	0.995	0.045
Tetanic tension after 5 minutes of rest period following fatigue protocol	0.038	0.997	0.046

All values have been expressed as Mean ± SD

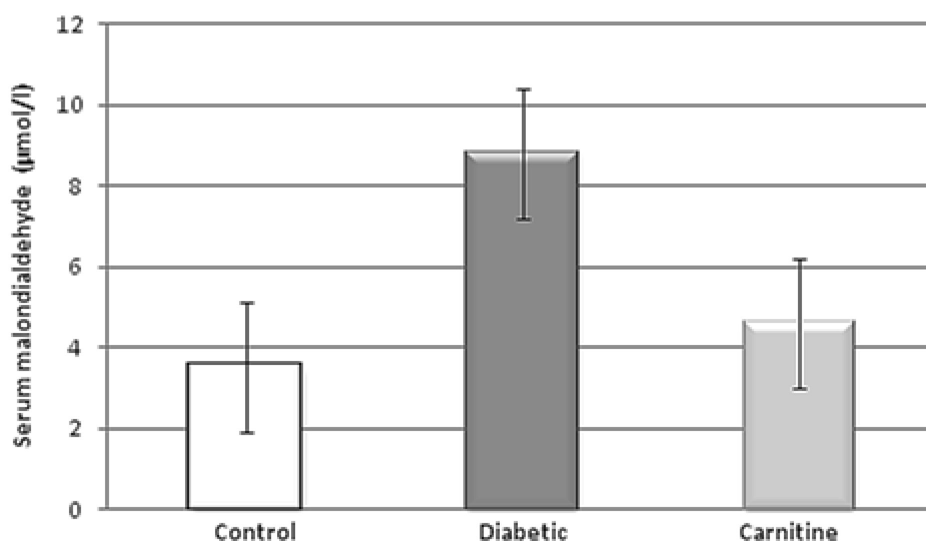

Figure 1: Serum malondialdehyde levels in three designated groups of rats at the 28th day of study
All values have been expressed as Mean ± SD; p value control vs diabetic 0.001; p value control vs carnitine 0.001; p value diabetic vs carnitine 0.001

Discussion

The animal model of T2DM developed by Srinivasan *et al*[16] was used in the present study because this model closely resembled the natural history and metabolic characteristics of human T2DM as compared to the other models that were based on feeding high sucrose or fructose diet. High fat diet was formulated in such a way that 59% of calories were provided through fat for inducing insulin resistance in rats over a short period of time.[23] β-cell destruction by low dose Streptozocin resulted in frank hyperglycemia.[16] The procedure was simple, less expensive and took short period to induce T2DM in rats. These rats also gained weight that was greater than the control group thus making it an ideal model of obesity induced T2DM similar to human model with increased body weight, plasma glucose and plasma triglycerides in diabetic rats.

The rats were subjected to 24 hours fasting after three weeks of preparation. Fasting has been shown to decrease the muscle glycogen content.[17] It was expected that decrease in muscle glycogen could be regained by normal rats (controls) while the diabetic rats were expected to remain deficient in muscle glycogen due to insulin resistance and deranged glucose metabolism.[1,2] Although the muscle glycogen content was not estimated in this

study, however, the decrease in maximal force of contraction at tetanizing frequency, extra decline in force of contraction during fatigue protocol and poor recovery from fatigue in muscles of the diabetic group as compared to the normal rats indirectly supported the expected decline in muscle glycogen.

Levo-carnitine in the dose of 200 mg per kilogram body weight of rats was administered to the carnitine group for six days after development of T2DM. The same dose was used by Cavassa in his study for the same duration. This dose was found safe in rats as it did not cause side effects even when administered for duration of one month. In healthy rats, this dose of *levo*-carnitine produced significant increase in forced muscular activity and endurance time.[11] *Levo*-carnitine was injected intraperitonealy to avoid the first pass metabolism by liver thereby ensuring optimum *levo*-carnitine levels in plasma and bioavailability in accordance with regimen documented by Hoppel *et al*[24] and Brass *et al*.[25]In our study, this dose of *levo*-carnitine led to increase in force of contraction at tetanizing frequency and decrease in fatigue in the carnitine group as compared to the diabetic group.

In present study, oxidative stress was measured by estimating the levels of MDA in serum. Serum MDA levels in control group of Sprague Dawley rats was 3.5 µmol/L which was consistent with the published data of different studies like Ruifen Zhang *et al* ,[26] Nevin Ilhan *et al*[27] and Feng-Xia *et al*.[28] Serum MDA was markedly increased in the diabetic group but decreased significantly in the carnitine group that suggested the antioxidant property of *levo*-carnitine. However, there still lies a significant difference between carnitine and control group. This manifests that the dose of *levo*-carnitine used in this study (200 mg/kg/day for 6 days) is insufficient to make the difference between serum MDA levels of control and T2DM rats insignificant.

Amin and Nagy administered *levo*-carnitine 250 mg/kg daily for 28 days orally to obese rats which significantly reduced the serum MDA levels (7.96 ± 0.08 µmol/L) as compared to diabetic rats (9.72 ± 0.08 µmol/L). However, serum MDA levels of *levo*-carnitine administered diabetic rats were significantly higher than control rats (4.69 ± 0.06 µmol/L).[29] Although *levo*-carnitine was given for longer duration in that study as compared to our study (6 days), but it was administered orally which resulted in below optimal serum *levo*-carnitine levels due to incomplete absorption and high first pass metabolism as compared to intraperitoneal administration which resulted in significantly higher MDA levels of *levo*-carnitine administered obese rats than control rats.[24,25] Malaguarnera *et al*[10] in a recent study found that use of *levo*-carnitine (2 g/day) in T2DM reduced the oxidative stress when given orally for three months. MDA and conjugated dienes were significantly decreased in patients manifesting the antioxidant action of *levo*-carnitine.

EDL muscle was selected for experimentation because it was believed to have abundant type II fibers which depend largely on glycolysis for adenosine triphosphate (ATP) production. Reduction in performance due to decreased glycogen content[2,3] and decreased activity of PDH is expected to be more evident in this muscle compared to muscles having type I fibers like soleus or mixed fibers like gastrocenemius.[3]

The maximum isometric twitch tension was not statistically different among the groups because the maximum force produced by the muscle depends on the number of myosin heads engaged with the actin filaments at a given point in time which in turn depends on the availability of the ATPs and calcium ions (Ca^{++}) in the sarcoplasm.[30]As the ATPs are adequately available in the sarcoplasm of the diabetic muscles and other factors like pH, phosphocreatine and free adenosine diphosphate (ADP) are found at optimal levels[31], therefore strength of contraction after single electrical stimulus was not adversely affected in the diabetic group as compared to the control.

The time to peak twitch tension shows the rapidity of release of Ca^{++} from the sarcoplasmic reticulum[18] which remained unaffected by the diabetic conditions as depicted by the data of our study. The time for relaxation to half of the maximum twitch tension signifies the rapidity of Ca^{++} pump to transport the Ca^{++} from the sarcoplasm into the sarcoplasmic reticulum and it remained unaffected due to adequate availability of ATPs in type II diabetic muscle.[31]

In study by Warmington *et a,l*[18] increase in time to peak twitch tension in single muscle twitches of EDL from genetically obese (*ob/ob*) mice as compared to healthy controls was observed. Similarly, half relaxation time was increased in *ob/ob* group as compared to control group. This increase was suggested to be due to the changes in muscle morphology with increased number of slow twitch fibers in the muscles of the *ob/ob* mice. Furthermore, probability of reduced Ca^{++} cycling ability of the SR in the *ob/ob* skeletal muscle was also mentioned. In this study, obesity was induced by feeding high fat diet rather than genetic manipulation. So, any significant difference in the fiber type of obese diabetic mice as compared to the controls was unlikely to be present. Thus, no change in the time to peak contraction and half relaxation time was observed in this study.

In another study by Toscano *et a,l*[32] the contractile properties of the soleus and EDL from rats at 12 month of age, whose mothers were undernourished during pregnancy and lactation, were studied. Strength of contraction was reduced in undernourished rats as compared to the controls due to decreased muscle mass and energy stores. Contraction time in undernourished group was decreased compared to control group while half relaxation time was decreased in undernourished group as compared to control group probably due to changes in calcium movement from the sarcoplasmic reticulum. Protein deprivation in these rats induced modifications in thyroid status which in turn led to reduced activity and concentration of Ca^{++} ATPase in sarcoplasmic reticulum. These changes resulted in

decreased duration of contraction and increased time in relaxation during single muscle twitch.

Although T2DM leads to protein catabolism but in this study, rats remained alive for one week after the induction of T2DM was confirmed. This small time period was probably insufficient to induce substantive reduction in muscle mass as compared to rats used by Toscano *et al*.[32] In a study by Pierce and Dhalla[33], no effect on Ca^{++} ATPase activity was observed in rat cardiomyocytes after 2 weeks of diabetes development. It is suggested that after 1 weeks of development of diabetes in our study, the Ca^{++} ATPase activity remained undisturbed in the skeletal muscles of the rats. So, no effect on the half relaxation time was evident in muscles of these rats.

Muscle fibers require large amount of ATP for tetanic contraction which was provided by the stored muscle glycogen especially during anerobic conditions[30]. T2DM is known to reduce the skeletal muscle glycogen by around 20%[2]. Furthermore, the rats in this study were subjected to 24 hours fasting which was known to significantly decrease the muscle glycogen levels as compared to the amount present in the skeletal muscle at the beginning of the fast.[17] Based on the results of present study, it can be deduced that the skeletal muscles of the normal rats were able to make up the deficit of glycogen stores when normal diet was resumed after 24 hours of fasting due to normal insulin activity. On the other hand, the diabetic rats were not able to rebuild skeletal muscle glycogen stores because the activity of glycogen synthase enzyme, responsible for the synthesis of glycogen, was impaired in T2DM due to the insulin resistance and relative insulin deficiency.[34] It is expected that combined effects of T2DM and 24 hours fasting might have resulted in significant reduction in the skeletal muscle glycogen. Furthermore, PDH activity is reduced in T2DM which impairs utilization of muscle glycogen as shown by Mondon *et al*[35] who documented a fourfold increase in lactate levels in association with a decrease in the active form of PDH in both fat stores and skeletal muscles in rats with T2DM.

The skeletal muscle contraction is also related with production of increased amount of reactive oxidative species which cause decline in muscle exercise performance[36] and level of these reactive oxidative species is also high in T2DM.[37] So, once the skeletal muscles of the control group were stimulated to produce tetanic contraction, they were able to generate maximum force of contraction of higher magnitude as compared to the skeletal muscle of diabetic group due to the better fuel supply and its utilization along with low oxidative stress and lactate levels.

In the study by Warmington *et al,*[18] increased maximum force of contraction at tetanizing frequency was observed in the *ob/ob* group as compared to the control group. The reduced Ca^{++} cycling ability of the sarcoplasmic reticulum in the *ob/ob* skeletal muscle was suggested as the cause of this difference by the authors. This resulted in generating increased amounts of tetanic force of contraction in the *ob/ob* muscles. In our study, the maximum force of contraction at tetanizing frequency was decreased in obese diabetic rats as compared to the control group that was suggestive of reduced ability to generate and utilize ATPs rather than increase in Ca^{++} recycling.

The maximum force of contraction in skeletal muscles of the carnitine group was almost similar to the control group. This indicated that skeletal muscles of diabetic group supplemented by *levo*-carnitine probably had adequate amount of ATP to generate optimum force during tetanic contraction. It had been documented that *levo*-carnitine improved insulin sensitivity in T2DM thereby increasing glucose uptake by all tissues up to 8% of the basal glucose uptake level in the absence of *levo*-carnitine.[38] It is proposed that increase in *levo*-carnitine levels in plasma led to increased storage of glycogen in the skeletal muscles.[39] *Levo*-carnitine also normalizes PDH activity and stimulates oxidative utilization of glucose and reduces lactate levels in plasma and skeletal muscles.[8] *Levo*-carnitine is also known to reduce the levels of ROS produced as a result of exercise[9] and T2DM.[40,41]

In the present study, muscle fatigue was measured as a decrease in maximum force of contraction due to the repeated contractions at tetanic frequencies. The force of contraction was significantly decreased more in the EDL of the diabetic rat group as compared to the control group. Once the muscles were stimulated repeatedly at the tetanic frequencies, the diabetic muscles considered to have reduced glycogen stores and increased lactate levels, exhausted their ATP supply at significantly greater rate than that of control group. Furthermore, quick depletion of phosphocreatine and decreased glycogen utilization had also been reported in diabetic muscles along with faster decline in the muscle pH as result of repeated contractions.[42] In the study by Warmington *et al*,[18] reduction in the fatigue of the muscles in ob/ob rats was attributed to the changed fiber type causing reduced reliance on anerobic glycolysis.

Muscles from the carnitine group had almost similar decline in the force of contraction after fatigue protocol as compared to the control group indicating replenishment of the glycogen stores of the muscles[38,39] as well as the reduction in the lactate levels[9] and ROS[10] in both the groups. The delay in fatigue observed in the *in vivo* study by Cavassa, although not identified by the author, was also probably due to increased oxidative metabolism of the fatty acids in the type I fibers.[11]

These studies compared the fatigue in rats given carnitine supplementation compared to the normal rats. In our study, the effects of carnitine supplementation on fatigue of the diabetic rats' muscles has been studied. The diabetic muscles manifested the greater decline in force due to deranged metabolism. It is suggested that *levo*-carnitine supplementation corrected the metabolic process of the diabetic muscles in our study, as already confirmed by the previous work, thus making the rate of decline in muscle force comparable to the control rats.

The recording at tetanic frequency after rest of 5 minutes also showed statistically significant decline in strength of

the diabetic group muscles. The diabetic skeletal muscles had been shown to have reduced capability of glucose uptake.[3] So, the muscles of the control and carnitine groups were able to uptake glucose from the buffer medium in which they were placed and adequately utilized it[40] but the diabetic muscles could probably do so at much reduced rate. This might have resulted in better replenishment of the ATP stores in control and carnitine groups as compared to the diabetic muscles.

Conclusion

Increased oxidative stress and decreased force of skeletal muscle contraction at higher frequency of stimulation (tetanization) in type 2 diabetes mellitus is likely to be responsible for the reduced work capacity and easy fatigability. *L*-carnitine supplementation reduced the oxidative stress and corrected the underlying metabolic processes in skeletal muscles which led to normalization of the contractile parameters of skeletal muscles at higher frequency of stimulation (tetanization). This is likely to restore the work capacity of these muscles.

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