INTRODUCTION
Levo-carnitine (L-3-hydroxytrimethylaminobutanoate) is an endogenous compound which helps in transportation of long chain fatty acids inside the mitochondria for their metabolism, stimulation of pyruvate and branched-chain amino acid oxidative metabolism, ketone body formation, adenosin triphosphate (ATP)/adenosin diphosphate (ADP) production and gluconeogenesis in liver and muscle.۱ Carnitine-dependent metabolic processes provide skeletal muscle with the ability to gain and use energy. The skeletal muscles cannot synthesize l-carnitine and depend on circulating free l-carnitine to maintain normal metabolism.۲ Serum l-carnitine insufficiency leads to disturbed muscle metabolism which is likely to reduce skeletal muscle performance.۳

Type 2 diabetes mellitus (T2DM) is one of the diseases in which levo-carnitine insufficiency may manifest۴ along with reduction in muscle glycogen۵ and mitochondrial dysfunction leading to impaired ATP production.۶ During exercise, the energy demand of skeletal muscle increases tremendously,۷ but the disturbed metabolic processes in T2DM may hinder the provision of adequate fuel to the exercising muscles resulting in reduce work capacity and easy fatigability in type 2 diabetic patients.

The present study was planned in a diabetic model developed in Sprague-Dawley rats to study serum levo-carnitine levels and contractile functions of rodent skeletal muscle at high frequency stimulation.

METHODOLOGY
This randomized control trial was carried out at Physiology Department, Army Medical College, Rawalpindi, from January 2009 to January 2010, on 60 healthy Sprague-Dawley rats (body weight 250 ± 10 grams). Approval from the Ethical Committee of Centre for Research in Experimental and Applied Medicine, Army Medical College was obtained. Rats were kept in 2 x 3 feet steel cages in a well ventilated room at 20 – 22°C with photoperiod of 12 hour light and 12 hour
all measured forces were normalized to muscle mass and expressed as Newton per gram (N/g) wet muscle mass. Statistical Package for Social Sciences (SPSS) version 17 was used to calculate mean with standard deviation of all variables. Statistical significance of differences between the groups was determined by independent sample t-test and p-value ≤ 0.05 was considered significant.

**RESULTS**

The body weight of rats was found to be higher in the diabetic group as compared to the controls. Serum l-carnitine levels reduced significantly (p < 0.01) in diabetic rats (30.29 ± 2.59 nanomoles / milliliter) as compared to the controls (39.41 ± 2.12 nanomoles/milliliter, Figure 1). This finding indicated the adverse effects of T2DM on serum l-carnitine levels.

There were no significant difference in MITT (p = 0.277), TPTT (p = 0.594) and 1/2RT (p = 0.138) [Table II] while significant difference was found in MFTT (p = 0.029).

### Table I: Body weight, plasma glucose and TG/HDL ratio between control and diabetic groups after three weeks.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>256.6 ± 7.40</td>
<td>269.70 ± 8.35</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.83 ± 0.51</td>
<td>23.13 ± 2.32</td>
<td></td>
</tr>
<tr>
<td>TG/HDL</td>
<td>1.37 ± 0.55</td>
<td>2.17 ± 0.78</td>
<td></td>
</tr>
</tbody>
</table>

All values have been expressed as Mean ± SD

Plasma glucose ≤ 16.65 mmol/l is considered normal

TG = Triglycerides; HDL = High density lipoproteins

### Table II: 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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum isometric twitch tension (N/g)</td>
<td>0.32 ± 0.04</td>
<td>0.31 ± 0.03</td>
<td>0.277</td>
</tr>
<tr>
<td>Time to peak twitch tension (ms)</td>
<td>19.6 ± 3.3</td>
<td>20.1 ± 3.9</td>
<td>0.594</td>
</tr>
<tr>
<td>Time taken to relax from maximum twitch tension to its 50% (ms)</td>
<td>20.5 ± 3.2</td>
<td>21.8 ± 3.5</td>
<td>0.138</td>
</tr>
</tbody>
</table>

All values have been expressed as Mean ± SD

### Table III: 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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group</th>
<th>Diabetic group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum fused tetanic tension (N/g)</td>
<td>4.000 ± 0.006</td>
<td>3.975 ± 0.061</td>
<td>0.029</td>
</tr>
<tr>
<td>Maximum fused tetanic tension after fatigue protocol (N/g)</td>
<td>1.813 ± 0.029</td>
<td>1.787 ± 0.054</td>
<td>0.020</td>
</tr>
<tr>
<td>Tetanic tension after 5 minutes of rest period following fatigue protocol (N/g)</td>
<td>3.968 ± 0.029</td>
<td>3.936 ± 0.074</td>
<td>0.031</td>
</tr>
</tbody>
</table>

All values have been expressed as Mean ± SD
TTFP (p = 0.020) and RF (p = 0.031) between the two groups (Table III). These findings manifested decline in muscle functions at high frequency of stimulation in T2DM while no significant effect was observed in single twitch parameters.

**DISCUSSION**

The animal model of T2DM developed by Srinivasan et al. was used in the present study as it was simple, less expansive and closely resembled the natural history and metabolic characteristics of human T2DM. Fifty nine percent of calories were provided through high fat diet which led to the increased levels of plasma triglycerides and increased availability of fatty acids for preferential use by oxidation. This reduced the insulin-mediated glucose uptake and utilization by skeletal muscles thereby inducing insulin resistance over a short period of time, as manifested by the high TG/HDL ratio of 2.17 (cut off value, 1.8) after 21 days. Administration of low dose sterptozocine led to the marked hyperglycemia in rats due to β-cell destruction and relative deficiency of insulin.

In the present study, serum l-carnitine levels were found significantly reduced in the diabetic rats (Figure 2). It has been documented earlier that total, free and esterified carnitines were decreased in diabetic patients. Free l-carnitine content was especially lower in these patients which could have impaired the fatty acid oxidation and resulted in elevated triglyceride and free fatty acid concentrations, reduced ketogenesis and lipid infiltration in liver and muscles. In response to decreased plasma l-carnitine levels, skeletal muscles release l-carnitine which leads to its depletion in the muscles. This reduces muscle's ability to use long chain fatty acids as metabolic fuel resulting in weakness, muscle aches, asthenia, malaise and early fatigue.

EDL consists of abundant type II muscle fibers rich in glycogen and depend largely on glycolysis for ATP production. The 24 hours fasting is known to decrease the glycogen content to one-third of its initial content in these muscle fibers. Although, the muscle glycogen content was not estimated in this study, however, decline in muscle performance of the diabetic group at optimum frequency indirectly suggests the regaining of muscle glycogen by healthy rats in controls whereas EDL of diabetic rats were expected to be glycogen deficient due to the insulin resistance and deranged l-carnitine metabolism.

MITT was found statistically similar in both groups because in T2DM decreased availability of glucose makes free fatty acids as the preferred substrate for skeletal muscles. This maintains ATP, pH, phospho-creatine and free ADP concentrations at normal levels in the skeletal muscle at rest. Adequate availability of ATPs enables engagement of optimum number of myosin heads with the actin filaments at a given point in time. This caused similar force production by the diabetic and control muscles after a single electrical stimulus.

TPTT depends upon the rapidity of Ca++ release from the sarcoplasmic reticulum (SR) while rapidity of the Ca++ pump to transport Ca++ from the sarcoplasm into the SR determines 1/2RT. Adequate availability of ATPs in resting diabetic muscle allows the Ca++ pump to perform optimally during the single muscle contraction, therefore, no significant difference was found between these two groups.

Warmington and colleagues observed an increase in TPTT and 1/2RT in single muscle twitches of EDL from genetically obese (ob/ob) mice as compared to the healthy controls. This increase was related to the greater number of slow twitch fibers along with the reduced Ca++ cycling ability of the SR in the ob/ob skeletal muscle. In this study, obesity was induced without any genetic manipulation by feeding high fat diet. So, there was no possibility of change in fiber type in obese diabetic mice, hitherto, no change in TPTT and 1/2RT was observed.

In a study by Toscano et al., the contractile properties of two muscles; soleus and extensor digitorum longus, were observed in sibling rats (age 12 months) of undernourished mothers during pregnancy and lactation. The strength of contraction was reduced in undernourished rats due to the decrease in muscle mass and energy stores. TPTT was decreased while 1/2RT was increased as compared to the controls because protein deprivation in these rats induced modification in thyroid status which in turn led to the reduced activity and concentration of Ca++ ATPase in SR. Protein catabolism is common in T2DM but short duration of our study was insufficient to induce substantive reduction in muscle mass as compared to over matured rats born to malnourished mothers used...
by Toscano et al. In a study by Howarth and colleagues, no effect on Ca++ ATPase activity was observed in rat cardiomyocytes during early stages of T2DM. It is suggested that after 7 days of development observed in rat cardiomyocytes during early stages of glycogen content, quick depletion of phosphocreatine, supply at significantly greater rate because of reduced -carnitine insufficiency leading to the exhaustion of ATP likely to be the result of metabolic derangements due to diabetic muscle was significantly reduced.

Thus, force of tetanic contraction produced by the skeletal muscles stimulated at tetanizing frequencies. These observations indicate that decreased -carnitine levels in insulin resistant T2DM did not significantly alter the metabolism in resting skeletal muscles. Thus, functional parameters of a single isometric muscle twitch were not significantly affected.

MFTT in diabetic group was significantly compromised as compared to the control group (Table III) because muscle fibers required a large amount of ATP for tetanic contractions which were believed to be provided by the stored muscle glycogen especially during anaerobic conditions. Induction of T2DM and 24 hours fast probably decreased the glycogen levels significantly in diabetic muscles. The skeletal muscles of the normal rats were able to make up the deficit of glycogen stores when diet was resumed while the diabetic rats were not able to do so due to -carnitine insufficiency and insulin resistance. Furthermore, oxidative metabolism of pyruvate and fatty acid utilization is impaired while oxidative stress is increased in diabetic muscles due to -carnitine insufficiency. These metabolic irregularities are likely to be responsible for impaired generation and utilization of ATPs by the contracting muscles. On the other hand, Warmington et al. observed increased MFTT in the ob/ob skeletal muscle due the reduced Ca++ cycling ability of SR as compared to the control.

These results indicate that due to the decreased -carnitine levels in insulin resistant T2DM, metabolic processes are unable to provide sufficient fuel to the skeletal muscles stimulated at tetanizing frequencies. Thus, force of tetanic contraction produced by the diabetic muscle was significantly reduced.

The greater decline in TTFP of the diabetic muscle is likely to be the result of metabolic derangements due to -carnitine insufficiency leading to the exhaustion of ATP supply at significantly greater rate because of reduced glycogen content, quick depletion of phosphocreatine, impaired fatty acid oxidation and increased lactate levels along with increased oxidative stress in the muscle. In the study by Warmington et al., delayed fatigue observed in ob/ob rats was attributed to the changed fiber type with reduced reliance on anaerobic glycolysis.

Metabolic derangements due to -carnitine insufficiency could be the most likely cause of poor RF in diabetic group because of reduced capability of glucose uptake and utilization by diabetic muscles. On the other hand, muscles of the control group replenished the ATP stores by adequately utilizing glucose from the buffer medium leading to better recovery from fatigue.

Hitherto, -carnitine insufficiency in diabetes mellitus resulted in early fatigability of muscles at tetanizing frequencies and these muscles were unable to recover optimally as compared to the controls.

CONCLUSION

Decreased serum -carnitine levels and force of skeletal muscle contraction at higher frequency of stimulation (tetanization) in type 2 diabetes mellitus probably result in reduced work capacity and easy fatigability.

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REFERENCES


