Effect of glucocorticoid on protein and creatine content of inactivated muscle of rats

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In denervation, there was loss of protein in gastrocnemius muscles and this loss was more in prednisolone treated animals. There was significant change of protein loss between tenotomy and tenotomy with prednisolone treatment. The reduction of muscle creatine was observed in denervation with prednisolone treatment. It was about 50% of the normal control group and about 40% when compared to other limb. In denervation alone, the creatine loss was about 24%. In tenotomy and in tenotomy with prednisolone treatment, the loss of creatine was also significantly high. All these figures regarding the reduction of muscle creatine in different experiments were highly significant. The reduction of muscle weight, protein and creatine content of muscle in denervation were due to inactivation of the muscle and due to trophic changes caused by loss of motor supply to the muscle. But in tenotomy, the reductions were only due to inactivation.

Keywords: Creatine, Denervation, Gastrocnemius weight, Glucocorticoid, Prednisolone, Protein, Tenotomy

Creatine is synthesized in the liver from methionine, glycine and arginine. In the skeletal muscle, it is phosphorylated to creatinephosphate, which is an important energy store for ATP synthesis. The creatine, which is available in urine, is formed from phosphocreatine. Creatine is normally excreted in urine in trace but creatineuria occurs normally in children, in women during and after pregnancy and occasionally in non-pregnant women. Creatineuria occurs in starvation, thyrotoxicosis, poorly controlled diabetes mellitus and various primary and secondary diseases of muscles.

Phosphocreatine is a high-energy compound that can be used by cells to drive energy requiring reactions. This compound is formed from creatine and ATP by the action of creatine kinase (CK), also called as creatine phosphokinase (CKP). The reaction performed by this enzyme is readily reversible. ADP and phosphocreatine can be converted into creatine and ATP. Increased levels of this enzyme in the blood have been found to be associated with number of serious seemingly related conditions including heart attack, alcoholism, muscular dystrophy, stroke, epilepsy and other neurological and endocrine disorders. In general, elevated serum CK levels are associated with conditions leading to tissue damage and death (necrosis).

Of 700 g total phosphorus in the body, 9% is present in skeletal muscle. Most intracellular phosphorus exists as organic compounds, such as creatine phosphate and adenosine monophosphate and triphosphate. Muscle creatine is increased by 39% after exercise training.

Though creatine, an essential component of the muscle, its quantity in muscle in different diseased conditions has not been adequately evaluated. Moreover the protein is most important component of muscle but how this protein is affected by denervation or tenotomy with or without simultaneous administration of glucocorticoid is also not adequately studied. Therefore the present work has been undertaken to estimate the creatine and protein in muscle which is normal or inactivated. The study is also extended to know the effects of glucocorticoids on the muscle creatine and muscle protein.

Materials and Methods

Animals — Male albino rats (50) weighing 100-110 g were purchased from local animal supplier.
and kept in separate cages. The animals were given water and laboratory diet ad libitum. They were divided in the following 5 groups of 10 animals each.

- **Group I – Control**
- **Group II – Denervation**
- **Group III – Tenotomy**
- **Group IV – Denervation with prednisolone treatment**
- **Group V – Tenotomy with prednisolone treatment**

*Tenotomy and denervation* — Tendoachelis was cut in one of the legs. The sciatic nerve was cut at the thigh in one leg each. During operation, the animals were under general anaesthesia by ether inhalation. Animals were sacrificed by ether anaesthesia after 30 days and the gastrocnemius muscles were collected weighed and homogenized with water (1:19).

*Drugs and chemicals* — Prednisolone and creatinine were purchased from Sigma Chemical Co, USA and picric acid, sodium tungstate, sulphuric acid and sodium hydroxide were from Glaxo Laboratories, India (ANLAR).

*Effects of glucocorticoids* — Prednisolone was dissolved in 10 ml water. One ml of the solution containing 1 mg prednisolone was given orally to each of the animal in group 4 and 5 once daily for 30 days.

*Estimation of creatine* — Creatine was estimated after its conversion to creatinine. For this freshly collected muscle was homogenized with distilled water (1:19). The homogenate (2 ml) was mixed well with 2 ml distilled water, 2 ml of 5% sodium tungstate and 2 ml of 2/3 \( N \) \( \text{H}_2\text{SO}_4 \). The whole mixture was centrifuged for 10 min at 2000 rpm. To 3 ml of the supernatant 1 ml of standard picric acid solution (containing 0.04 mg/ml picric acid) was added and heated for 45 min in boiling water. Then, it was cooled and 1 ml of 0.75 \( N \) \( \text{NaOH} \) was added. Similarly, blank and standard were prepared. Readings were taken in spectrophotometer at 520 \( \mu \text{m} \) wavelength. One gram of creatinine was formed from 1.16 g of creatine.

The estimation of protein was done from the homogenate by the method of Paul et al. Permission from the animal ethical committee was taken before the beginning of the work.

Student’s *t* test was used for statistical analysis. *P* values <0.001 were considered very highly significant.

### Results

In group I the protein and creatine content in the muscle of left and right side showed insignificant variation. But both the protein and creatine in the muscle were very significantly reduced in tenotomised and denervated muscles (Tables 1 and 2).

These reductions were more when prednisolone was administered to the animals. All these reductions were highly significant (*P*<0.001) but highest reduction occurred in denervation with prednisolone treatment in group IV.

Creatine is an important constituent of muscle tissue, the normal level being 450 mg/100g. This level is decreases in various muscular disorders. A reduction has also been noted after steroid myopathy. The structural and functional integrity of muscle cells is largely dependent on creatine content. A reduction

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Protein (g%)</th>
<th>Creatine (mg /100 g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control non-operated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left side</td>
<td>19.97 ± 0.097</td>
<td>448.5 ± 7.592</td>
</tr>
<tr>
<td>Right side</td>
<td>20.21 ± 0.137*</td>
<td>450.9 ± 5.836*</td>
</tr>
<tr>
<td>II. Denervation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-operated side</td>
<td>19.86 ± 0.060</td>
<td>44.50 ± 4.947</td>
</tr>
<tr>
<td>Operated side</td>
<td>16.52 ± 0.085**</td>
<td>338.2 ± 4.563**</td>
</tr>
<tr>
<td>III. Tenotomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-operated side</td>
<td>19.74 ± 0.112</td>
<td>456.6 ± 6.010</td>
</tr>
<tr>
<td>Operated side</td>
<td>18.16 ± 0.083**</td>
<td>393.9 ± 3.799**</td>
</tr>
<tr>
<td>IV. Denervation with prednisolone treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-operated side</td>
<td>19.74 ± 0.081</td>
<td>402.0 ± 6.253</td>
</tr>
<tr>
<td>Operated side</td>
<td>14.66 ± 0.103**</td>
<td>243.2 ± 5.422**</td>
</tr>
<tr>
<td>V. Tenotomy with prednisolone treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-operated side</td>
<td>19.56 ± 0.136</td>
<td>405.7 ± 2.970</td>
</tr>
<tr>
<td>Operated side</td>
<td>17.52 ± 0.104**</td>
<td>346.4 ± 4.415**</td>
</tr>
</tbody>
</table>

*P* values: *>0.05; **<0.001

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Reduction (%) of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle weight</td>
<td>Muscle protein</td>
</tr>
<tr>
<td>1. Tenotomy</td>
<td>16.08</td>
</tr>
<tr>
<td>2. Tenotomy with prednisolone treatment</td>
<td>16.23</td>
</tr>
<tr>
<td>3. Denervation</td>
<td>13.93</td>
</tr>
<tr>
<td>4. Denervation with prednisolone treatment</td>
<td>41.46</td>
</tr>
</tbody>
</table>
of creatine in denervated muscles was also noted by Catheart et.al\textsuperscript{11} and Banker and Denny Brown\textsuperscript{12}, but there are scanty reports of the creatine and protein content of denervated and tenotomised muscle under the influence of glucocorticosteroid analogues such as prednisolone. In the present study, an attempt has been made to evaluate the effect of prednisolone on the creatine and protein content of denervated and tenotomised gastrocnemius muscle in albino rats.

Most of the creatine in the body is found in muscle as creatine phosphate. When the muscle works, creatine phosphate provides the energy through hydrolysis of its high energy phosphate bond, forming creatine and phosphate in the form of ATP. This reaction is reversible and catalysed by the enzyme creatine phosphokinase\textsuperscript{9}.

**Discussion**

The creatine content of normal muscle tissue in albino rats was about 450 mg\% while in inactivated muscle it was reduced to 339 mg\% in denervated muscle and 394 mg\% in tenotomised muscles. On administration of prednisolone the creatin was further reduced. All the reductions were highly significant (Tables 1 and 2).

Both endogenous and exogenous glucocorticoids caused steroid myopathy. It can be of sufficient severity to impair ambulation. Steroid can produce myopathy in respiratory muscle in asthmatic persons. The recovery from the steroid myopathy may be slow and incomplete\textsuperscript{13}. Weakness is common in Cushing syndrome and is usually associated with proximal muscle wasting, including wasting of gluteus maximus and arm muscle mass. Many such patients cannot rise from squatting position without assistance\textsuperscript{10,13,14}. Muscle weight in denervated and tenotomised gastrocnemius was significantly reduced when compared to normal values. But this reduction was more when compared with untreated animals. Myofiber atrophy is the final outcome of muscle weakness induced by catabolic factor like glucocorticoid. In vitro study showed that this catabolic reaction is based on myotube atrophy. Hydroxyl radical formation in skeletal muscle is responsible for myopathy by dexamethazone\textsuperscript{15}. Apoptosis of the muscle cells may occur on denervation and in steroid myopathy\textsuperscript{16,17}, which may be the cause of excess protein and creatine loss from the muscles in the present experiment. Dexamethazone treated denervated muscle showed marked Myosin Heavy Chain (MHC) loss as well as there was decreased level of m-RNA of MHC1 (Myosin Heavy Chain 1)\textsuperscript{18}.

Weight loss, myofiber atrophy and loss of creatine kinase was noticed after denervation\textsuperscript{19,20} in rat extensor digitorum longus and soleus muscles. Donoglu et.al\textsuperscript{21} reported that Ca\textsuperscript{2+} handling proteins in muscle are changed after denervation and the density of capillary network is reduced due to denervation which can be attributed to the changes of muscle protein\textsuperscript{22}. The report of creatine in denervated muscle is scanty. Literature is also almost silent about the creatine content of muscle during and after the treatment with glucocorticoid analogues like prednosolon, dexamethason etc.

Recently it has been reported that creatine has the potential to increase cellular hydration and myogenic transcription factors and facilitate the up regulation of muscle specific-genes such as myosin heavy chain, possibly leading to muscle hypertrophy\textsuperscript{23}. Besides creatin supplement can increase the weight and functions of the reinervated muscle\textsuperscript{24}.

**References**


