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*Original Article*

# Histological changes of the skeletal muscle due to muscle tension: A study in rats

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**Abstract**
**Objective:** To analyze the influence of muscle tension in the histological changes of the skeletal muscle. **Methods:** Fifty-four Sprague-Dawley (SD) rats were randomly divided into 3 groups. The left gastrocnemius muscles of the rats were dissected with only the neurovascular pedicles intact; the tibial nerves were cut and immediately repaired by epineurial suture. Then the Achilles tendons were isolated and treated accordingly; the Achilles tendon were lengthened by 0.5 cm in lengthened group, shortened by 0.5 cm in shortened group and left alone in normal (control) group. In the 2nd, 4th and 8th weeks after operation, the specimens were taken from gastrocnemius muscle for histological study by light and electron microscope. **Results:** In comparison between the groups, the gastrocnemius muscles in the shortened group showed less severe muscle atrophy and connective tissue proliferation, bigger diameter and cross section area of the muscle fibre than those in the normal and lengthened groups in all the postoperative periods. **Conclusion:** A proper high tension of the muscle may decrease the muscle dystrophy and stops the histological changes of skeletal muscle by having much longer fibre length and bigger cross sectional area. Also increases the muscle function as the muscle active force-generating range is determined by muscle fibre length, while maximum muscle force is determined by physiologic cross-sectional area.

**Key Words**: Tension, Skeletal Muscle, histological changes, myofiber dystrophy

Clinically, a neurovascularized free muscle transfer is an ideal solution to the reconstruction of a devastated skeletal muscle or a paralyzed extremity [1,2]. However, histopathological changes of muscle is inevitable also the most challenging task. In literature, many reconstructive surgeons emphasized the need of placing the transferred muscle in an optimal tension. It remains unknown how a skeletal muscle responds towards the tension in terms of histological changes as the muscle active force-generating range is determined by muscle fibre length, while maximum muscle force is determined by physiologic cross-sectional area.[3]

## Materials and Methods

Fifty-four Sprague-Dawley (SD) rats were used in the experiment. The left gastrocnemius muscles of the rats were dissected with only the neurovascular pedicles intact; the tibial nerves were cut and immediately repaired by epineurial suture. Then the Achilles tendons were isolated and treated accordingly. The rats were randomly divided into 3 groups with 18 rats in each, based on the manner of the Achilles tendon treatment. In group A (the normal group), the Achilles tendons were just isolated without cut, in group B (the shortened group), the Achilles tendons were cut and sutured back with a shortening of 0.5 cm, and in group C (the lengthened group), the Achilles tendons were divided with stair cut and sutured back with their length increased by 0.5 cm. The wounds were then closed and the rats were allowed to move freely.

At 2nd, 4th and 8th weeks after operation, 6 rats from each group, the gastrocnemius muscle specimens were taken for histological study by light microscope (Olympus, Japan) with HE stain and electron microscopic (JEW-200 CX, Olympus, Japan) study respectively. The cross section area and diameter of myofiber of the gastrocnemius muscle were measured with the assistance of computer. All the data were analyzed by the software SPSS 11.0 for Windows

## Results

### 1. Diameter and cross section area of the myofiber

In the very beginning, the diameter and cross section area of the gastrocnemius muscle fibre decreased in all groups as the time went on, being the least at the 4th postoperative week, but increased gradually as the reinnervation of the muscle proceeded (Table 2, 3).

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They were found increased at the 8th week after operation in all groups. Observed in the same time period, the cross section area and diameter of the muscle fibre differed in different groups. The difference in cross section area and diameter of the gastrocnemius muscle fibre were significant between the shortened group and the lengthened group, with the *P* value being 0.002 and 0.004 at the 2nd week, 0.015 and 0.01 at the 4th week, 0.013 and 0.05 at the 8th week. The differences in cross section area and diameter of the gastrocnemius muscle fibre were not statistically significant between the normal group and the lengthened group, between the shortened group and the normal group at all time periods, with an exception of the difference of the diameter of the gastrocnemius muscle fibre between the normal group and the lengthened group, which had a *P* value of 0.035.

**Table 1:** The diameter of the gastrocnemius muscle fibre (μm)

| Groups | Time | | |
|---|---|---|---|
| | 2nd week | 4th week | 8th week |
| Lengthened | 11.26±1.83 | 6.48±1.81 | 7.53±1.78 |
| Normal | 12.18±1.73 | 7.86±1.94 | 10.2±1.86 |
| Shortened | 12.83±1.61 | 9.31±1.85 | 11.82±1.67 |

**Table 2:** Cross section area of the gastrocnemius muscle fibre ($\mu m^2$)

| Groups | Time | | |
|---|---|---|---|
| | 2nd week | 4th week | 8th week |
| Lengthened | 625.76±72.29 | 475.49±65.21 | 576.48±75.79 |
| Normal | 728.32±68.46 | 512.48±46.08 | 592.86±83.56 |
| Shortened | 786.12±72.52 | 589.12±71.15 | 647.16±46.49 |

**2. Histological changes of the muscle**

**Under Light Microscope:**

As the tibial nerve was cut, although repaired immediately, the gastrocnemius muscles in both groups underwent denervation and demonstrated muscle atrophies of different extents. Mesenchymal connective tissue proliferations were also seen under the light microscope. At the 4th week, both the muscle atrophy and the connective tissue generation seemed to be the most severe. Since then, as the sutured tibial nerves generated and the gastrocnemius muscles were reinnervated gradually, the muscle atrophy was improved consequently. However, there did some differences in muscle fibre atrophy between the two groups. Even in 4th postoperative week, the muscle atrophy and mesenchymal connective tissue hyperplasia seen in the cross section of muscle fibre ( HE×100 ) in the shortened groups were less severe than those in both the normal and the lengthened groups **(Figs. 1-3).**

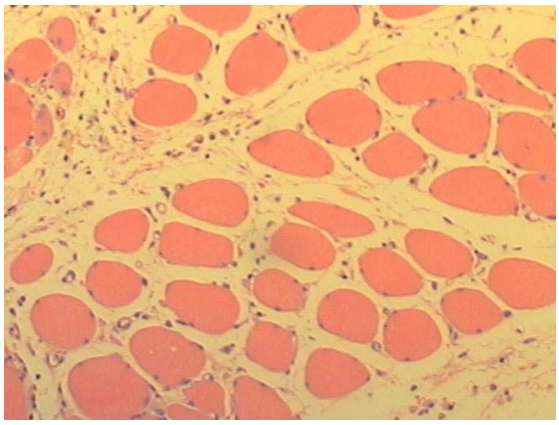

**Fig 1:** Cross section of muscle fibre severe muscle fibre atrophy and hyperplasia of mesenchymal connective tissue. 4th week (HEX100) the lengthened group

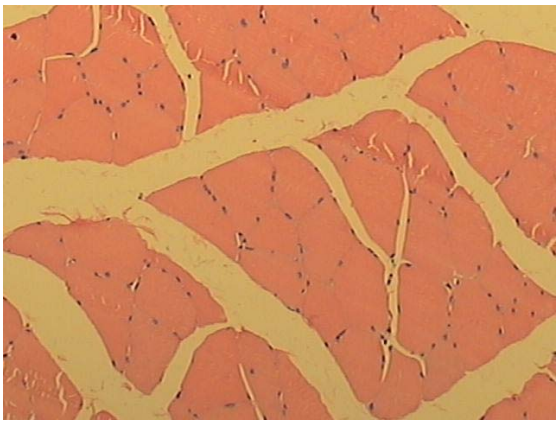

**Fig 2:** Cross section of muscle fibre, light muscle fibre atrophy and proliferation of connective tissue. 4th Week (HE X 100) the shortened group

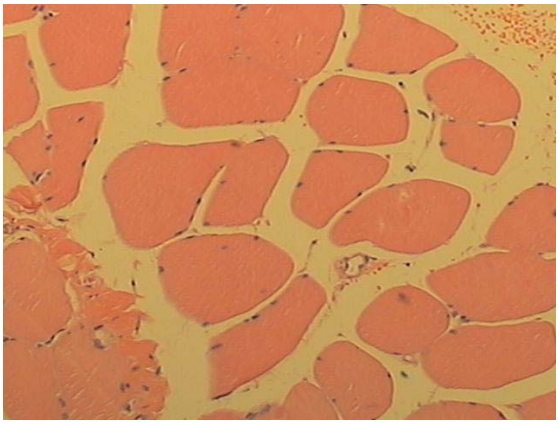

**Fig 3:** Cross section of muscle fibre
Less severe muscle atrophy and hyperplasia of mesenchymal connective tissue. 4th week (HE X 100) the normal group

**Fig 4:** Oedema, fatty changes and muscle fibre disorderedly distributed clearly seen 4th week (EM X 3000) the lengthened group

**Under Electron Microscope**

The histological changes seen under electron microscope are similar with that light microscope. Each group went through dystrophy of muscle in different extend but the most severe is seen in the 4th week and less severe in the 8th week. The lengthened group got the most severe dystrophy that other groups and the shortened group got the less severe dystrophy with more and enlarged muscle fibre and multiple mitochondrial cristae. The muscle fibres seen disorderly distributed, oedema between parenchyma cells, clear fatty tissue, oedema of mitochondria in different extend.

**Fig 5:** Less Oedema and muscle fibre distribution less disorderedly clearly seen 4th week (EM X 3000) the normal group

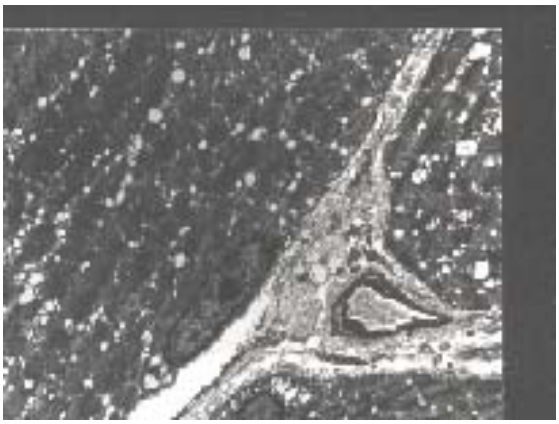

**Fig 6:** Oedema and muscle fibre distribution is much less disorderedly than other groups. 4th week (EM X 3000) the shortened group

**Discussion**

The morphological structure of the tissue is the basis for the function of tissue organ[4]. When denervated, a muscle will certainly have its morphological structure changed as it loses the nutrition provided by the nerve. In this study the tibial nerves in all groups had been cut and repaired immediately by direct suture. All the gastrocnemius muscles presented with typical muscle atrophy after denervation. However the extent of atrophy differed time to time and group by group.

Before the fourth postoperative week the muscle atrophy in the same group increased as the time went on, but then gradually improved as a result of the regeneration of the sutured tibial nerves. This study revealed that there existed some relationship between the tension that the muscle force and the histological change that the muscle presents. Although the differences in cross section area and diameter of the gastrocnemius muscle fibre were not statistically significant between the normal group and the lengthened group, between the shortened group and the normal group at all time periods, but the difference in cross section area and diameter of the gastrocnemius muscle fibre were significant between the shortened group and the lengthened group, with the *P* value being 0.002 and 0.004 at the 2nd week, 0.015 and 0.01 at the 4th week, 0.013 and 0.05 at the 8th week. This finding has specific importance in term of clinical practice. It means that in performing a neurovascularized free muscle transplantation if the transplanted muscle is fixed under a properly high tension it may have better functional recovery following its reinnervation as the muscle active force-generating range is determined by muscle fibre length, while maximum muscle force is determined by physiologic cross-sectional area[3]. The ultrastructural changes seen under the electron microscope is similar with the light microscope being the severe changes seen on the lengthened groups and the least severe changes seen in the shortened groups. In the 4th week the changes seen is the most severe than other time groups.

Muscle Tension also increases in the number and length of sarcomeres and also the new muscle tissue[5,6] so increases the function. Illizarov[7] observed the changes in muscle during bone lengthening under the electron microscope and reported that by the influence of tension-stress effect, muscle fibre become enlarged with multiple mitochondrial cristae and with hypertrophy of their nuclei, and that elongation of the muscle occurs not only by myofibrillogenesis in preextisting muscle fibre but also by the formation of new muscle tissue characterized by an increased number of satellite cells, the appearance of muscle myoblasts, and their fusion into myotubes. Rantanen[8] found that immobilization in a relaxed position led to a significantly more extensive fibre atrophy in the calf muscles than tensioned positions in rat.

**Conclusion**

This study has revealed that tension of the muscle can influence the histological changes of muscle. To keep a suitably high tension on the muscle with its nerve cut and repaired immediately can reduce its atrophy extent, increase the length and cross section area of the muscle fibre. It is therefore suggested that, in order to get better functional recovery, a transplanted muscle should have proper high tension intraoperatively, and an injured limb should be immobilized in such a position that a high tension can be put on the paralyzed muscle after its nerve repair

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