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# The effect of tibial lengthening on gastrosoleus muscle function: an electrophysiological study

## *Gastrosoleus kas fonksiyonu üzerine tibial uzatmanın etkileri: Elektrofizyolojik çalışma*

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**Amaç:** Deneysel tibial uzatma sırasında gastrosoleus kas grubunda gerilmeye bağlı meydana gelen kas kasılma kuvvetlerindeki değişiklikler elektrofizyolojik olarak değerlendirildi.

**Çalışma planı:** Ağırlıkları 600-800 gr arasında değişen 22 kobay (guinea pig) üzerinde distraksiyon histiogenezi uygulandı. Kobayların sağ tibialarına, semisürküler eksternal fiksatör uygulanmasından sonra osteotomi yapıldı ve latent periyot beklenmeden 15 gün boyunca günde iki kez 0.25 mm hızla distraksiyon gerçekleştirildi. Hayvanlar rastgele iki gruba ayrıldı. Bir gruba eksternal fiksatöre sabitlenen ve plantar fleksiyonu engelleyen ayak pedalı eklendi. Kontrol grubundaki deneklerin ayak bilek hareketleri serbest bırakıldı. Distraksiyonun 5, 10 ve 15. günlerinde posterior tibial sinir, sinir uyarıcısı ile sabit frekansta uyarıldı. Gastrosoleus kas kontraksiyonları elektriksel uyarım çeviricisi ile ölçülerek kaydedildi.

**Sonuçlar:** Beşinci günde, deney grubunda ölçülen ortalama kas kasılma kuvvetleri kontrol grubuna göre anlamlı derecede yüksek bulundu ($p<0.05$). Onuncu günde, deney grubunda kas kasılma kuvvetlerinde hızlı bir düşüş saptandı; 15. günde, başlangıç değerlerine göre kas kasılma kuvvetlerinde %81 oranında azalma vardı ($p<0.05$). Bununla birlikte, kas kuvvetleri başlangıçtan sona kadar kontrol grubundan yüksekti ($p<0.05$). Deney grubunda 10. günden sonra kas kasılma kuvvetlerindeki düşüşün yavaşladığı ve gastrosoleus fonksiyonunun daha iyi korunduğu izlendi. Kontrol grubundaki deneklerin ayak bileklerinde giderek ilerleyen ekinus deformitesi gelişti. Bu grupta da kas kasılma kuvvetlerinin deney süresince giderek azaldığı görüldü ($p<0.05$).

**Çıkarımlar:** Klinik uygulamada, tibial uzatma sırasında ekinus deformitesinin engellenmesi ve ayak bileği fonksiyonlarının sağlanması için gastrosoleus kompleksinin kuvvetinin korunması; bu amaçla, uzatma sırasında kasın kademeli gerilmesinin sağlanması gerekmektedir. Kasta kısalık varsa veya kasta gerilme ve deformite bekleniyorsa, distraksiyon osteogenezisinden önce önlem alınması ve kas boyunun korunması önem taşımaktadır.

**Anahtar sözcükler:** Kemik uzatma/yöntem; elektrofizyoloji; ekin deformitesi; eksternal fiksatör; kobay; kas, iskelet; osteogenez, distraksiyon; rejenerasyon; tibia.

**Objectives:** We evaluated the changes in electrophysiological characteristics of the contraction forces of the gastrosoleus complex due to stretching in experimental tibial lengthening.

**Methods:** Distraction histiogenesis was performed in 22 guinea pigs weighing 600 to 800 g. Following the application of a semicircular external fixator and right tibial osteotomy, distraction was applied at a rate of 0.25 mm two times a day for 15 days without a latency period. The animals were randomized to two groups. In the study group, a foot plate preventing ankle plantar flexion was affixed to the external fixator, while ankle motions were unrestricted in the control group. On days 5, 10, and 15, the posterior tibial nerve was stimulated with a nerve stimulator at a constant frequency. Gastrosoleus muscle contractions were measured with a transducer and contraction forces were recorded.

**Results:** On day 5, muscle contraction forces measured in the study group were much higher than the control group ($p<0.05$). On day 10, however, muscle contraction forces showed a rapid decline in the study group and, at the end of the study, muscle contraction forces decreased by 81% compared to the baseline values ($p<0.05$). Yet, throughout the study period, muscle contraction forces were always higher than the control group ($p<0.05$). In addition, the rate of the decrease in muscle strength slowed down after day 10 in the study group, and gastrosoleus function and strength were much better preserved. Equinus deformity developed progressively in the ankles of the control animals whose muscle contraction forces also showed significant decreases during the experiment ($p<0.05$).

**Conclusion:** In clinical applications of tibial lengthening, the strength of the gastrosoleus complex should be preserved to prevent equines deformity and maintain ankle functions. This can be achieved through gradual stretching of the muscle during distraction. If there is shortening before surgery or muscle stiffness is expected during lengthening, measures should be taken before distraction osteogenesis and muscle length should be preserved.

**Key words:** Bone lengthening/methods; electrophysiology; equinus deformity; external fixators; guinea pigs; muscle, skeletal; osteogenesis, distraction; regeneration; tibia.

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Distraction histiogenesis is accepted as an effective method in extremity lengthening and new bone regeneration. However, muscles,vessels and neuronal structures are seen to be the limiting factors. Contractures and muscle weakness occur due to restricted adaptive response of muscles to the lengthening. Despite the controversies in literature, general opinion is that the muscle fibers grow and elongate during distraction histiogenesis. The tension stress applied to the bone not only stimulates the bone but also stimulates neogenesis in soft tissues.[1-6]

Development of equines deformity in tibial lengthening is a common problem seen in many of the experimental and clinical applications. Many measures are applied to prevent this condition.[7-12] Although there are many studies about responses and reactions of muscles and neuronal tissues to the stretching during distraction histiogenesis, clinical studies about the effects of equinus deformity on the gastrosoleus complex is very rare.[8]

The goal of this study is to evaluate the changes in muscle contraction and electrophysiological characteristics of the gastrosoleus muscle due to stretching in experimental tibial lengthening model.

## Materials and methods

This study was performed on 22 Guinea pigs weighting 600-800 gr at the time of surgery. All animal procedures comformed to national regulations and approval of experimental animal studies committee before the study.

### Surgical protocol

50 mg/kg cefazolin sodium ( SEFAZOL® Mustafa Nevzat İlaç Sanayi, İstanbul, Türkiye) was given intramuscularly 30 minutes before surgical intervention for infection prophylaxis. For the purpose of anesthesia, 8 mg/kg xylazine hydrochloride (ROMPUN®; Bayer Healthcare, Leverkusen, Germany) and 100 mg/kg of ketamine hydrochloride (KETALAR®, Pfizer USA) was applied intramuscularly to the animals. Following the standard surgical preparation, a semicircular external fixator designed for experimental extremity lengthening in small rodents was applied to the diaphysis of the right tibia of the subject with the use of 1,2 mm threaded pins.[13] With the use of anteromedial approach, the bone was exposed at the level of distal tibio-fibular junction and the bone osteotomised.

Guinea pigs were divided into two groups. In the experiment group special foot plate was assembled to the distal end of the external fixators for the purpose of preventing the ankle motions and these foot plate was kept in place during the experimental process. Rest of the animals separated as control group and they had unrestricted ankle motions during experimental period. Without waiting latency period, starting from the first postoperative day, lengthening was commenced in all animals with the rhythm of 0,25 mm twice a day for 15 days.

### Electrophysiology

Posterior tibial nerve stimulation was used to assess the electrophysiological properties of gastrosoleus muscle. For this purpose at the days 5,10 and 15 of distraction, the animals were given anesthesia which was used at the surgical intervention and no:2 nonabsorbable nylon sutures were applied to the achilles tendon where it attaches to calcaneus and the suture endings were connected to the mechanical transducer. Posterior tibialis nerve was found percutaneously with the use of unipolar electrode.The nerve was stimulated with a pulse of 10 ms duration at 50-Hz in gradually increasing voltages (Grass S8800 electrical stimulator, USA). Electrical potentials obtained were recorded using a polygraph recorder( Grass model 7D,USA). Values of maximum contraction forces

**Table 1.** Mean values of muscle forces of the groups

| | | Mean muscle force (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 5 | | | Day 10 | | | Day 15 | | |
| | | Experimental group | Control group | *p* | Experimental group | Control group | *p* | Experimental group | Control group | *p* |
| Stimulus | 1 | 6.0 | 1.1 | <0.05 | 2.5 | 0.3 | <0.05 | 0 | 0 | <0.05 |
| (volt) | 2 | 13.0 | 1.3 | <0.05 | 5.5 | 0.7 | <0.05 | 2.4 | 0.5 | <0.05 |
| | 3 | 19.1 | 1.9 | <0.05 | 5.8 | 2.4 | <0.05 | 3.6 | 0.8 | <0.05 |

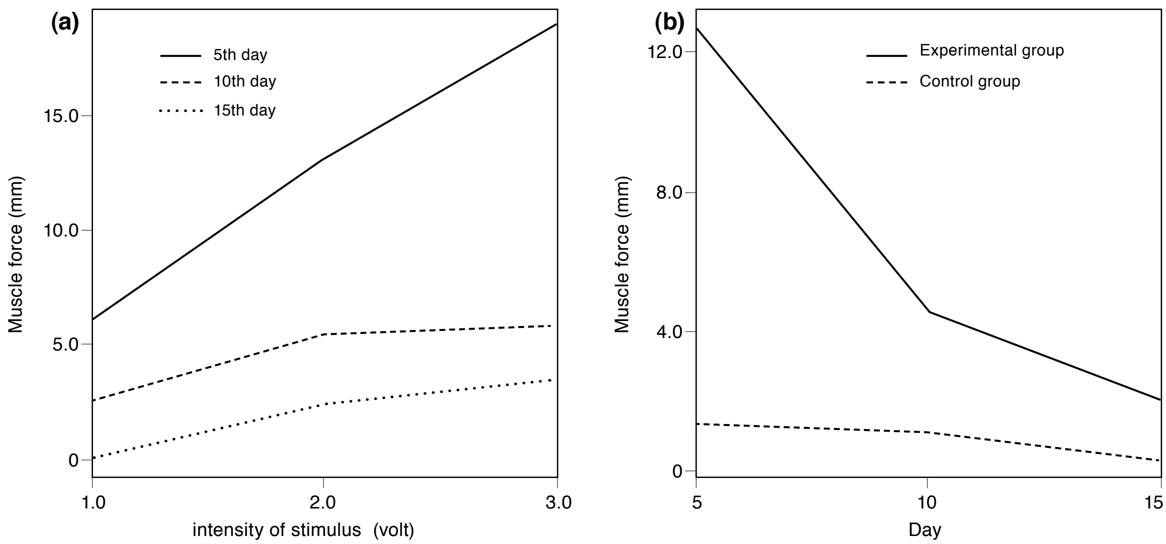


**Figure 1.** **(a)** Dmean muscle forces acording to days in experimental group **(b)** comprasion of mean muscle forces of the groups with 2 volt stimulus

obtained were used for statistical analysis. Kruskal–Wallis variant analysis for inter-group assessment and Mann–Whitney U test (Bonferroni correction) analysis for intra-group assessment was used to analyze the data. The data sets that differed with a probability of p<0.05 were considered significantly different.

## Results

In the experimental group, one of the subjects died from respiratory failure at the second day of distraction. Data was collected from the remaining 21 subjects. Superficial infection was seen in two subjects of the experimental group and in one subject of the control group which was not needed treatment . No another complication was seen besides from these. At the 5th day of distraction osteogenesis, mean muscle contraction force measured in the experimental group were found significantly higher than the control group (at 1,2,3 volts muscle contraction forces in experimental group is 6.0, 13.0, 19.1 mm and for control group is 1.1, 1.3, 1.9 mm; p<0,05) (Table1). In the following measurements at 10th and 15th days of distraction osteogenesis, mean contraction forces obtained from experimental group tended to decline rapidly, reaching a level far below the initial measurements. In this group, there was a 81 % decrease of muscle contraction forces at the end of study compared with initial measures (p<0.05) (figure 1). However, when the muscle forces of both groups which was obtained during the distracion were assessed ,these values were significantly higher in experimental group than the control group from beginning to the end of study (p<0,05) (figure 2).But after the 10th day of distraction, it was seen that the decrement of muscle contraction force slowed down and the function of gastrosoleus muscle was preserved much better in experimental group.

During the experiment, progressive equinus deformity developed in all subjects of the control group and there was progressive decrease in muscle contraction force in this group as seen in the experiment group (figure 3).For instance, the value of muscle force was 1.9 mm at the 5th day and 0.8 mm at the 15th day when stimulated with 3 volts (p<0,05). ). Because of the initial values were lower in control group than the

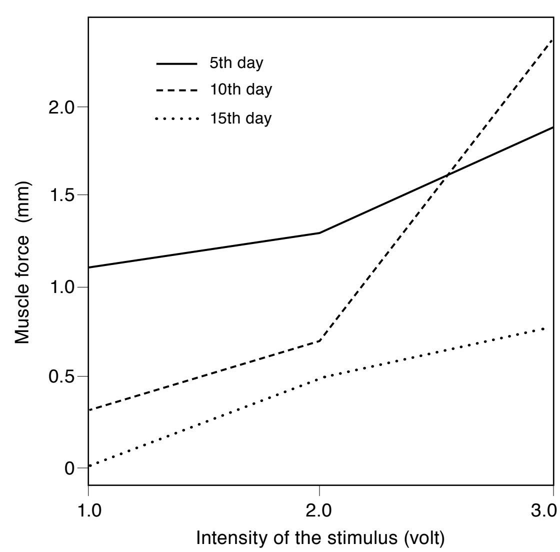


**Figure 2.** Mean muscle forces of the control group according to days

experimental group , reduction rate of muscle force tended to be lower than the experimental group (%57,8) (figure 3).

## Discussion

Adequate muscle and neuronal function and adaption of these tissues to the new extremity length following bone distraction is a prerequisite for a successful lengthening procedure. Muscle groups on one side of the cruris segment, especially posterior and medial surface, are much more resistant to lengthening than the antagonist muscles on the opposite surface.[13] During the tibial lengthening, remaining of the ankle at the plantar flexion position provides gradual elongation of the dorsiflexors and results in stretching and contracture of the plantar flexors. To investigate the response of muscle tissues to stretching, different animals varying from big mammals to small rodents are used in experimental studies. Small rodents which are good candidates to reflect the myosin isoform transformation are more preferred because of their low cost and ease of application.[13]

Parallel to the clinical practice in humans, in our study equinus deformity has developed in animals with their ankles freed during lengthening . It is reported that increased resistance to stretching of posterior muscles is a result of the anterior muscle groups' myosin heavy chain isoform rates being higher than the posterior group.[8,13] In clinical practice, many measures such as physiotherapy, weight bearing, casting, achill tenotomy, usage of different orthosis or night splints and fixation of the ankle to the tibial fixator are taken to prevent the development of equinus deformity during tibial lengthening.[8]

In this study, the foot plate was added to the distal ring of the external fixator and it was aimed to put the ankle in neutral position. Even though not fixing the knee joint is a factor affecting the function of the gastrosoleus muscle complex, the same factor applies for the control group, thus its affect is neglected. Furthermore, during their life span, small rodents in laboratory conditions pass their time on four legs , keeping their knees in a position near to complete flexion, and change of their knee position is much more limited than other mammals. This makes the additional fixation of the knee joint unnecessary. Also in our study, posterior muscle compartments were not affected by the pins of external fixator, so gastrosoleus muscle complex provided the necessarily conditions for the standard electrophysiological assessment.

Not always the same and well-matched results are obtained during the experimental studies analyzing the muscle changes during distraction histiogenesis. In some of these studies, edema, fibrosis and necrotic changes in muscle fibers were reported to be minimal while in the other studies, significant myogenic changes were observed.[14-16] Similarly, there are inconsistent results about the regenerative muscle changes such as hypertrophy and hyperplasia. [14,17-19] Although varied results exist, it is generally accepted that muscle fibers enlarge and elongate during distraction histiogenesis. A complication arousing from extremity lengthening is that surrounding soft tissues can not respond to the stretching as well as bone does. Although there are not many studies investigating how preventing equines deformity effects gastrosoleus muscle complex, number of studies about the changes in muscle and nerve as a response to stretching during distraction osteogenesis is gradually increasing. Mechanism of the neuropathic and myopathic injuries caused by tension, and their possible repair and adaptation mechanisms are not well known. Furthermore it is accepted that the changes which occur in muscle and nerves due to elongation depends on speed, rhythm and frequency of lengthening.[14,20-25] On the other hand, Makarov et al [20] showed that application of external fixation and osteotomy without distraction results in no detectable change in surrounding muscle tissue. In our study ,effect of fixation of ankle in neutral position on gastrosoleus muscle complex were investigated during distraction histiogenesis. Prevention of equines deformity and maintaining the stretching tension to the muscle tissue from the beginning of lengthening resulted in better preservation of the muscle function. Despite the prevention of ankle deformity ,muscle contraction responses progressively decreased in proportion with the amount of lengthening. Freeing the ankle and leaving the muscle devoid of tension and lack of biologic stimules as a result of this from the beginning has resulted in a sudden and huge decrease in muscle contractility.

Application of continue stretching force causes muscle tissue elongation and enlargement. This is due to increase of the sarcomeres in a serial manner along the muscle fiber, which is different than parallel augmentation of sarcomeres observed in muscle hypertrophy following exercise.[13,26-28] Caiozzo et al [26] pointed out that sarcomere length needs to exceed a threshold for the sarcomerogenesis to start during distraction osteogenesis. These researchers have hypothesized that possible cellular and molecular mechanisms may start the sarcomerogenesis at a certain stage of lengthening determined by the threshold length of the sarcomere. For example, the daily 0,5 mm lengthening of rat soleus muscle does not cause an increase in number of sarcomeres until sarcomere length reaches 2.6 $\mu$m, whereas it is shown that sarcomere length do not exceed 2.7 $\mu$m after the 8th day but an increase in the number of sarcomeres begins.[26] There are many studies supporting these results and describing the adaptation of the muscle during lengthening procedure as an event dependent on factors such as speed , rhythm and time of lengthening.[29-31] Tamai et al,[12] in their experimental distraction osteogenesis model, showed that continuation of the stretching tension on elongated sarcomeres for more than one week cause them to return to their previous length. In our study, as a result of preserving the length of gastrosoleus muscle by fixation of ankle and providing the elongation, despite the rapid decline in muscle force in first 10 days of distraction, as seen in figure 3 ,speed of decline slowed down by the progressing days. The similar effect was seen in the control group, but because in this group significant part of contraction forces were lost at the initial phase, stated more mildly. These findings support the opinion that influence of regeneration seen in muscle is initiated by a particular trigger mechanism and associated with the amountof lengthening

In clinical applications of tibial lengthening in order to prevent the equines deformity and maintaining the ankle functions, the strength of the gastrosoleus complex should be preserved. This study showed the importance of gradual simultaneously and same amount lengthening of muscle with bone during distraction to the cruris segment preventing the equines deformity and preserving the muscle force .To achieve this gradual stretching of the muscle from the beginning of the bone lengthening is needed. If there is shortening in the posterior muscle group of cruris prior to surgery or muscle stiffness or shortening is an expected adverse effect during lengthening the countermeasure should be carried out before distraction osteogenesis

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