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Evaluation of the muscle atrophies occurring after open surgical tenotomy and percutaneous tenotomy

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SUMMARY

The trial was designed to compare the atrophies occurring after open surgical tenotomy and percutaneous tenotomy using the experimental model that we established. Fourty Ross-800 hybrid chickens were divided into two equal groups. The first and the second group underwent open surgical tenotomy and percutaneous tenotomy of the Achilles tendon, respectively. The animals were sacrificed after eight weeks, and the wet weight, volume, height, diameter, rate of biomechanical elongations and strength of muscles were measured by precision measurement instruments. H & E staining was performed for the histopathological assessment of muscles. In addition, the preparations were photographed and the rate of fatty infiltration and the muscles were assessed by the pixel counting method. All data were analyzed using the Mann-Whitney u test.

Morphological evaluation showed that loss of muscle diameter in the surgical tenotomy group was statistically significantly different to that of the percutaneous tenotomy group ($p < 0.05$). The results of the histopathological evaluation and the counting process showed that there were significant results supporting the morphological findings and objectively showing an increase in fatty tissue in Group 1 ($p < 0.05$).

The decrease in the amount of elongation of the muscle at the time of rupture and an increase in power that was applied in Group 2 after the biomechanical study support the presence of atrophy and fibrosis and were found to be statistically significant ($p < 0.05$). In this experimental study, atrophy was created in both groups at the end of eight weeks. The amount of atrophy in Group 1 was found to be higher than that of Group 2. Histopathological findings and pure muscle tissue were assessed objectively with the novel pixel counting method.

Key words: Atrophy – Tenotomy – Pixel counting method

INTRODUCTION

Muscle atrophy, a significant orthopedic disorder that has been underestimated for a long time, also defined as the shortening of muscle fiber length without a change in the number of muscle fibers, results from various pathological conditions, such as hunger (Busquets et al., 2002), prolonged bed rest (Bloomfield, 1997), the removal of the mechanical load, denervation, immobilization or decreased gravity (Beuge and Aust, 1978), age (Picquet et al., 1988), and vari-

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ous pathological conditions, such as sepsis, chronic renal failure, diabetes, chronic heart failure, chronic obstructive pulmonary disease and cancer (Coutinho et al., 2004). Although a microscopic examination of the muscle atrophies of the subjects was included in the trial models, morphological and biomechanical changes in the muscle atrophies were not elaborated. In this experimental study, quantitative and morphometric measurements of the atrophic changes occurring in the gastrocnemius muscle of the subjects after the incision of the Achilles tendons in two different models were compared.

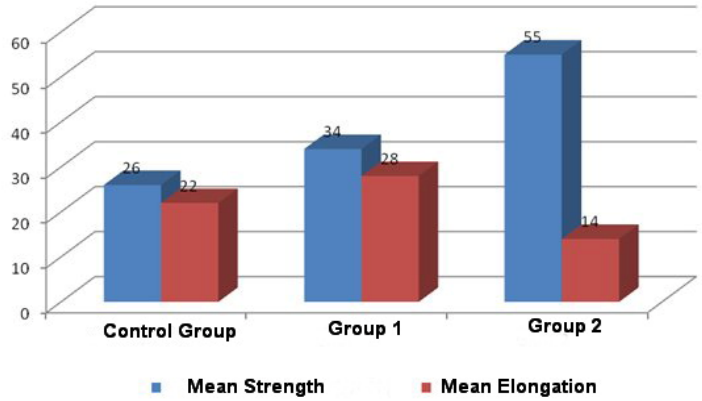


Fig. 1. Mean strength and elongation of muscles in the control group, Group 1 and Group 2 at the time of rupture after power was applied at a constant velocity of 20 m/sec.

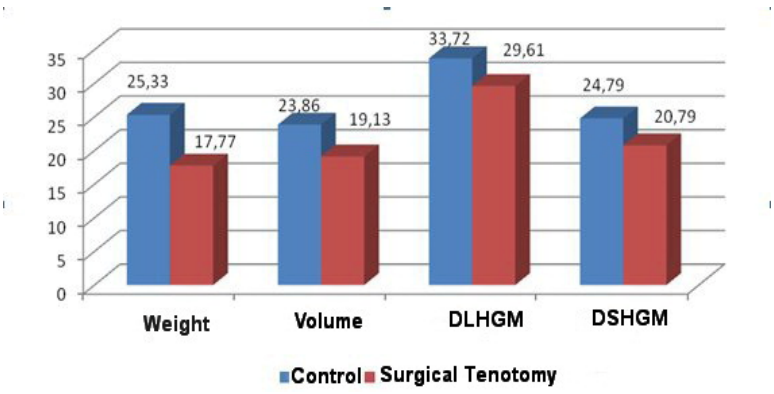
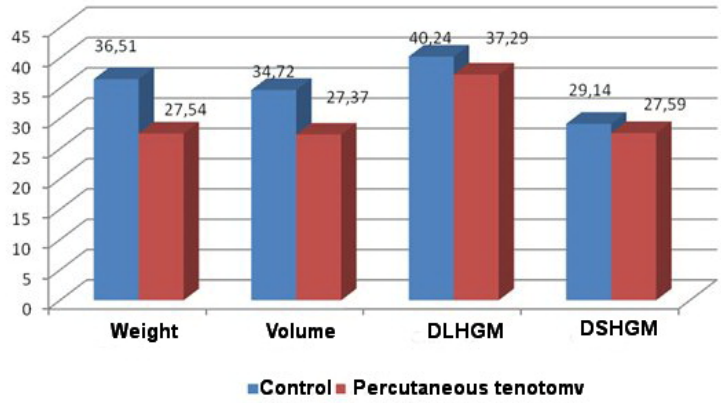


Fig. 2. A comparison of the mean weight, volume and diameter of the gastrocnemius muscle in the Control Group and Group 1.



MATERIALS AND METHODS

Forty Ross-800 type hybrid chickens weighing between 850 and 1200 grams were used in this study. The chickens were equally divided into two groups: the open surgical tenotomy group (Group 1) and the percutaneous tenotomy group (Group 2). Both groups were anesthetized with 50 mg/kg ketamine HCl (Alfamine®) and 10/mg/kg xylazine (Alfazyne®) and tenotomy of the left lower limbs was performed. A single dose of 1cc of gentamicin (80 mg/cc) and metamizole sodium (5 mg/kg) was administered intramuscularly to the chickens undergoing surgery.

Postoperatively, no immobilization was performed and they were free to move.

Open surgical tenotomy group (Group 1)

After the anesthesia, a cutaneous incision of approximately 2 to 3 cm was made longitudinally over the Achilles tendon. It was dissected from surrounding soft tissue and exposed, and cut transversely with scalpel number 20 and left as it was. The surgical site was closed with cutaneous suture without suturing the tendon sheath.

Percutaneous tenotomy group (Group 2)

The Achilles tendon was palpated proximal to the ankle of the chickens through the skin surface. It was cut with scalpel number 20 in a way to be perpendicular to the skin and the Achilles tendon without the soft tissues being dissected.

The surgical area was closed by cutaneous suture and freed.

The experimental animals were sacrificed by cervical dislocation after they were anesthetized with ketamine HCl in week 8.

A total of 20 subjects were randomly and equally divided into two subgroups: Group 1 (A-B) and Group 2 (C-D) for morphological and biomechanical studies before they were sacrificed.

Fig. 3. A comparison of the mean weight, volume and diameter of the gastrocnemius muscle in the Control Group and Group 2.

After they were sacrificed, both of the hind legs of the subjects were disarticulated from the hip joint. After the gastrocnemius muscle was freed, wet weights were measured with precision balance (g/100), then the diameter of the thickest part of the bellies, and long and short head of the muscles were measured and the muscles were put into a beaker with ml/100 accuracy and the volume of the muscles was measured by the amount of overflow of the normal saline.

Histopathological evaluation

Transverse and longitudinal sections from the thickest parts of both of the heads of the gastrocnemius muscle, flap-shaped sections from the musculotendinous junction and transverse sections from the tendon were taken and they were all stained with H&E. Preparations were evaluated by the same pathologist under a microscope. Fatty infiltration, degeneration, fibrosis, vascularization and the inflammatory response of the muscles were evaluated on the sections.

The sectional area where the fatty infiltration and degeneration were predominant was evaluated with the smallest magnification (4 x 0.10). Adipose tissue (white area) and muscle tissue (red area) were identified by their colors in digital images taken under an Olympus B * 51 branded microscope from this cross-sectional area, and these colored dots (pixels) were counted using the pixel counting method.

All measurements were evaluated statistically with the Mann-Whitney U test.

Biomechanical evaluation

During the biomechanical study, approximately 1cm of the tips of the long head of the gastrocnemius muscle and Achilles tendon were fixed into the machine (Shimadzu Autograph AG-IS—10 kN, Kyoto, Japan), and force was applied to the muscle up to breaking point with the constant velocity of 20 m/sec. The resistance of the muscle at the time of rupture and the amount of elongation of muscle fibers were measured, and the amount of force applied until the moment of rupture of the muscle – the amount of the elongation of the muscle fibers are shown with Figure 1.

RESULTS

The resistance of the muscle at the time of rupture, and the amount of elongation, volume, weight and diameter of muscle fibers were measured with the biomechanical study. Tables 1 and 2 and Figures 1-3 show the mean values and standard deviations obtained from the measurements.

The higher rate of loss of diameter of both heads of the gastrocnemius muscle in Group 1 compared to Group 2 was statistically significant ($p < 0.05$) (Table 1, Figures 2 and 3).

The obtained data were statistically compared with the control groups, and all the p-values of Groups 1 and 2 were found to be lower than 0.05 with the Mann-Whitney U-test analysis.

When each of the three groups was examined, the higher rate of the mean strength in Group 2 compared to the Control Group and Group 1 was found to be statistically significant. Accordingly, the higher rates of mean elongation in the Control Group and Group 1 compared to Group 2 were also found to be statistically significant ($p < 0.05$) (Table 2).

Loss of elasticity took place in Group 2 due to a lower amount of elongation. Resistance to applied power occurred as a result of the loss of elasticity.

Table 1. Mean percentages of the areas that underwent atrophy in Group 1 and Group 2

	Group 1 (mean ± SD)	**Group 2 (mean ± SD)**	**p**
Weight (mg)	-29.17 ± 8.06	-24.49 ± 5.58	0.232
Volume (ml)	-19.82 ± 7.46	-20.73 ± 4.82	0.999
DLHGM (mm)	-11.95 ± 5.86	-7.35 ± 1.34	0.040*
DSHGM (mm)	-15.72 ± 8.70	-5.83 ±1.38	0.006*

Mann-Whitney u test. Statistically significant p value (P <0.05) . DLHGM Diameter of the long head of Gastrocnemius muscle. DSHGM Diameter of the short head of Gastrocnemius muscle

Table 2. Mean strengths and elongations in the Healthy (Control) Group, Group 1 and Group 2.

	Healthy Group 1	Group 1	Group 2	p
Strength (N)				**0.002°***
Mean	31.67± 6.47	37.36± 6.02	49.17± 12.14	0.613[1]
				0.002[2]*
Min-Max	22.96-40.78	30.78-48.12	35.00-67.96	0.038[3]*
Elongation (mm)				**0.001°**
Mean	23.33±2.89	25.54±4.59	14.96±4.67	0.889[1]
				0.002[2]*
Min-Max	20.82-27.78	19.33-32.15	8.56-21.21	0.001[3]*

Kruskal-Wallis test, Mann-Whitney u-test Group 1: Surgical tenotomy group. Group 2: Percutaneous tenotomy group. Statistically significant p value (*P <0.05). ° Between three groups; [1] Control and surgical tenotomy; [2] Control and percutaneous tenotomy; [3] Surgical tenotomy and percutaneous tenotomy.

Histopathological findings

Vascularity, degeneration, fatty infiltration, inflammation and fibrosis were evaluated with a histologic examination, and these parameters were rated as no (3), mild (2) and severe (1).

The higher rate of vascularity, degeneration, fatty infiltration, inflammation and fibrosis in the transverse and longitudinal sections of both heads of the gastrocnemius muscle in Groups 1 and 2 compared to the Control Group was found to be statistically significant ($p < 0.05$).

In the Achilles tendon specimens, the higher rate of degeneration in Groups 1 and 2 compared to the Control Group, the lack of severe vascularity in Group 1 compared to Group 2, and the slightly higher rate of fatty infiltration in Group 1 compared to the Control Group were statistically significant ($p < 0.05$).

After the adipose tissue (white area) and muscle tissue (red area) were identified by their colors in digital images taken under a microscope while evaluating the fatty infiltration, the results of the pixel counting (Ceylan, 2010) were arranged in Table 3.

For measuring the volume of pure muscle tissue, the mean morphological volume of the groups and the rate of the muscle tissues (red area) in the histopathological sections that were obtained by the pixel counting method were used. While this value was 13.49 ml in the Control Group in Group 1, it decreased to 7.79 ml in the open surgery group. While this value was 19.62 ml in the Control Group, in Group 2 it was decreased to 14 ml in the percutaneous surgery group. When the obtained results were compared, while the rate of the loss of the pure muscle tissue (contractile tissue) was found to be 42% in Group 1, it was found to be 28% in Group 2.

The lesser amount of pure muscle tissue in Group 1 compared to Group 2 indicated that the amount of atrophy was greater in Group 1. In addition, the rate of the loss of pure muscle tissue (loss of contractile elements) was found to be higher than the loss of the total muscle tissue. This indicated that the amount of atrophy in the true contractile muscle tissue was higher than that of the atrophy observed morphologically.

The results can be summarized in the following points:

1. Larger amount of muscle atrophy developed after the surgical tenotomy when compared to the percutaneous tenotomy.

2. The rate of fatty infiltration and degeneration was higher in the surgical tenotomy group.

3. Although the amount of elongation of the muscle decreased after percutaneous tenotomy, the resistance of the muscle against the applied force increased.

4. Although the rates of volumetric loss after tenotomy were similar in both groups, the rate of loss of the pure muscle tissue was higher in the surgical tenotomy group due to the higher amount of fatty infiltration.

Table 3. Mean percentages of the red-white areas in the Control Group, Group 1 and Group 2 according to pixel counting *

	Control group (n 9)	GROUP 1 (n 9)	GROUP 2 (n 9)
Red area (%)	6.53	40	47.57
White area (%)	41.86	54.41	49.60
* Other (%)	1.61	5.09	2.83

* Because colors other than red and white indicate structures other than adipose and muscle cells in the pixel counting method, they were defined as other.

DISCUSSION

The principal functions of the skeletal muscles are movement, postural behavior and respiration. Striated muscles create atrophy models that negatively affect quality of life through various physiological (aging) and pathological stimulations (tendon cuts, disuse and denervation).

In this study, histo-morphological and biomechanical comparisons of the atrophies of the gastrocnemius muscle occurred after controlled surgical incisions and percutaneous incisions of the Achilles tendon were performed.

Rabbits, rats, sheep and chickens were used as experimental models for the evaluation of the atrophies (Tate et al., 1983). In the study conducted by Robert et al., the chickens were selected as study subjects as the higher rate of the atrophy in the white muscle fibers than the red muscle fibers, the fatty infiltration in muscle tissue could be evaluated, and the technique is easily applicable in terms of physical size (Robert et al., 1973).

The atrophy model was created by performing a controlled tendon cut 1 cm proximal to the insertion site of the supraspinatus tendon of rabbits (Fabis et al., 1998). Meyer et al. (2005) used the atrophy model (uncontrolled) after partial or total rupture of the tendon of the supraspinatus at the basis of degeneration in humans. In this study, the atrophy model was achieved after the incision (controlled), which was performed by surgical dissection just proximal to the site where the Achilles tendon adhered to the calcaneus in the chickens in Group 1, while it was achieved after the percutaneous incision (uncontrolled) in Group 2. Immobilization was not applied to the chickens

after surgical intervention.

In the study of Zarzhevsky et al. (2001) comparing the effects of the immobilization atrophy on young and older rats and recovery after remobilization, it was found that the rate of loss of the muscle was found to be 52% in older rats and 49% in younger rats. In the study of Melis et al. on humans, fatty infiltration of the supraspinatus muscle was found to be severe in only 1% of the patients under 50 years of age, and it was observed that this ratio could increase up to 12% in adults older than 60 years (Melis et al., 2010). The same study showed that a younger age provides good protection against the development of fatty infiltration. Therefore, the study subjects were selected from among young animals.

In the study conducted by Sunderland et al. (1950) including different animal species, they emphasized that the atrophy occurred earlier (at a rate of 60%) in some rabbit and rat species in the first 30 days, and the atrophy occurring within the same period of time in monkeys and cats changed more slowly (at a rate of 10-40%). In the study of Barton et al. (2005) including mice, it was determined that atrophy of the muscles occurred rapidly (13% when compared to the healthy group) after tenotomy and this rate of the muscle mass was preserved for a subsequent four weeks. In our study, when the experiment was terminated after eight weeks, the rates of weight loss compared to the healthy sides in Groups 1 and 2 were 25.08% and 24.33%, respectively. When the volume losses were compared, the rates of loss of volume in Groups 1 and 2 were found to be 19.82% and 20.91%, respectively.

In the study conducted by Ceylan (2010), the rates of loss of the weight and volume of the gastrocnemius muscle were found to be decreased by 42.5% and 40.5%, respectively, in chickens after three weeks of external immobilization. Ceylan (2010) detected that the rates of loss of the weight and volume of the gastrocnemius muscle were found to be 54.4% and 57.5%, respectively, in the denervation atrophy model occurring after the transection of the sciatic nerve in the same study. In this study, the rates of the loss of the weight and volume were found to be similar to each other; both groups' lost percentages were found to be comparable in both groups. A lower rate of the loss of weight and volume suggested that the Achilles tendon healed by clinging to the soft tissues after the incision, and in this manner, progression of the atrophy was prevented in the late period.

In the study conducted by Fabis et al. (1998) on rabbits, the rate of fatty degeneration was found to be below 25% by using Goutallier classification (a classification made according to sections taken under computed tomography). In the study of Meyer et al. (2005) on the human supraspinatus muscle showed that fatty infiltration following full-thickness tendon transections was not distributed homogeneously in the muscle. Meyer et al. (2005) have shown that there was more fatty infiltration in the muscle areas close to the bone. There were also findings supporting this situation in this study. In both groups, while severe fatty infiltration (62.5% and 77%) was developed in the area close to the origin of the muscle, mild fatty infiltration (37.5% and 55%) was developed in the middle section of the muscle.

The fibrosis that was considered to be a change in connective tissue was found to be mild with the rate 55% in the surgical tenotomy group (Group 1). Cases of severe fibrosis were not found. In the percutaneous tenotomy group (Group 2), while mild fibrosis was detected with the rate of 62.5%, cases of severe fibrosis were not found. The lack of severe fibrosis was considered to be due to muscle functions being regained in the early period.

The importance of the presence, number and activation of the satellite cells in preventing the process of muscle degeneration and re-gaining normal function of the muscle is known (Kosar and Demirel, 2004; Le Grand and Rudnicki, 2007). In the study of Ceylan (2010), it was reported that a higher rate of the regeneration capacity of the denervation group than the immobilization group during the atrophic process, and higher rate of the loss of volume and weight in denervation group than the immobilization group may be due to the intracellular mechanism.

In this study, the ongoing presence of satellite cells has probably supported the lesser extent of atrophy.

When a comparative evaluation of the atrophies of diameters of the short and long heads of the gastrocnemius muscle was performed, the rate of the atrophy was found to be 15.72% in the diameter of the short head and 11.95% in the diameter of the long head in Group 1. This rate was found to be 5.36% in the diameter of the short head and 7.31% in the diameter of the long head in Group 2. Then, the atrophy in Group 1 was found to be greater than that of Group 2. While these findings were supported by the histological examination of the long head of the gastrocnemius muscle, no difference was found in the rates of fatty infiltration and degeneration in the transverse and longitudinal sections of the short head.

The study conducted by Barton et al. (2005) on mice showed that the passive tension of the musculotendinous region increased. In this study, mild (75% and 77%) degeneration occurred in the

musculotendinous regions of both groups. A significant increase in vascularity was observed in the musculotendinous region of both groups. In the study conducted by Jozsa et al. (1990) investigating the effects of immobilization and remobilization on the tendon, the amount of edema, splitting, fragmentation, and irregularity, and the diameter of the collagen were found to be decreased (degeneration and fibrosis) in the tendon after immobilization. In this study, while mild fibrosis was detected in all of the tendons of both groups, the rate of the severe degeneration was 50% in Group 1 and 66% in Group 2. Unlike the study of Ceylan (2010) on the immobilization and denervation atrophies in chickens, severe degeneration of the tendon was detected in the atrophy models occurring after tendon injury. The rates of degeneration in the musculotendinous region were similar.

With the histopathological examination, the areas where the rate of the fatty infiltration and degeneration was highest were evaluated by small magnification after the samples were stained with H&E. The values given in the evaluation were completely subjective. Toraman and Türkoglu (2009) used a method similar to the method used in the study, called the automatic detection of the required cell area in histopathological images. In this study, the mean rate of the white area was found to be 41.86%, 54.41% and 49.60% in the Control Group, Group 1 and Group 2, respectively. The rate of red areas was found to be 56.53%, 40% and 4757% in the Control Group, Group 1 and Group 2, respectively. As it is seen here, the rate of fatty infiltration in Group 1 was found to be higher than Group 2, and the amount of muscle in Group 1 was found to be lower than Group 2.

In the study of Ceylan (2010), the rate of loss of pure gastrocnemius muscle tissue was detected to be 2/3 in subjects that underwent immobilization, and 3/4 in the subjects that underwent denervation. In this study, the rate of loss of volume of pure gastrocnemius muscle tissue was found to be 42% and 28% in Group 1 and Group 2, respectively. After surgical and percutaneous tenotomy conducted on chickens for eight weeks, the rate of the empirical decrease in pure muscle tissue was found to be (motor strength) 2/5 and 1/4 in Group 1 and Group 2, respectively.

In this study, the amount of elongation of the muscle up to the moment of rupture was measured by applying force at the velocity of 20 m/sec. There were no significant differences between the elongations in Group 1 and the Healthy Group. However, the rate of the resistance of the muscle against applied force increased by 17% due to the atrophy. In Group 2, the rate of elongation of the muscle decreased by 35.84% and the rate of the resistance of the muscle against applied force increased by 55.24% when compared to the Control Group. The lower rate of resistance in Group 1 was attributed to the amount of fatty infiltration, and degeneration was less obvious in Group 1 than in Group 2 with histopathological examination. The rate of the resistance of the muscles that underwent atrophy was found to be higher due to the decreased length of the muscles. This biomechanical study supports both views.

This study allowed the performing of morphological, histopathological and biomechanical comparisons of the atrophy models that are frequently used in literature. Morphological, histopathological and biomechanical changes in the muscles in the surgical tenotomy group (Group 1) were found to be more statistically significant than the atrophy in the muscles in the percutaneous tenotomy group (Group 2).

The authors are of the opinion that these findings are useful in obtaining objective data about untreated tendons and delayed muscle tendon repairs, and the capability of contraction and gliding after muscle transplantation.

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