

Electric Stimulation Versus Active Exercises in Prevention of Muscle Atrophy Histological Study

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ABSTRACT

To determine the effect of electric stimulation and active exercise on prevention of muscle atrophy in simple physiological immobilization, this study was conducted on thirty albino rats for one month. Their body weight ranged between 120-150 grams at the beginning of the study. The rats were classified randomly into three groups of equal numbers. Control group, electrical stimulation group and exercise group. The all rats were immobilized by a plaster cast covering the whole right lower limb keeping the ankle & hip joint are free. In electrical stimulation group window was opened on the front of the thigh over quadriceps muscle to apply electrical stimulation. Daily isometric exercise for quadriceps muscle were performed by walking or running on a treadmill for 10 minutes. The change in muscle fiber size in each of the three groups was compared with each other after daily treatment by electric stimulation or exercises to determine the effect of electric stimulation and exercise therapy on prevention of muscle atrophy. The results of this study strongly support that active exercise had a greater effect on prevention of muscle atrophy than other group.

Key Words: Muscle, Atrophy, Exercise, Electric Stimulation

INTRODUCTION

Muscle atrophy is a common complication of immobilization^{1,6}. The role of physical therapy during immobilization is to keep the skeletal muscles and their physiological effects on blood circulation around normal level. Active exercises and electrical stimulation are the most important therapeutic tools in any physical therapy clinic. These therapeutic techniques are still of need to build up their scientific background. The aim of this study was to determine the effect of electric stimulation and active exercise versus

placebo on prevention of muscle atrophy in simple physiological immobilization.

MATERIALS AND METHODS

Design

It was 2 x 1 pre test - post test research design. Electric stimulation and active exercises were the independent variables, and the muscle fiber size was the dependent variable.

Sample

Thirty albino rats was used in this experiment. Their body weight ranged between 120-150 grams at the beginning of the study.

They were randomly and equally grouped into three groups.

Group 1: (Control group). Ten rats were immobilized by a plaster cast covering the whole right lower limb, started above the forefoot and ended below the hip joint, keeping the ankle joint and hip joint free from immobilization. The period of immobilization continued for one month without any treatment.

Group 2: (Electric stimulation group). Ten rats were immobilized (same as in group 1), but a window was opened in the front of the thigh over the quadriceps muscle to apply daily treatment. Electrical stimulation was given for this muscle during immobilization for 10 minutes for one month.

Group 3: (Active exercise group). Ten rats were immobilized (same as in group 1). Daily isometric exercises for the quadriceps muscle were performed by running on a treadmill for about 10 minutes for one month.

Equipment

1. A unit for low-frequency electrical stimulation "Endomed 581". It has triangular pulsed current for stimulation of denervated muscle fibers. Point electrodes 5 mm were used for stimulation.

Specification of the current used in the study

- Current type: Triangular pulsed current.
- Phase duration: 0.02 ms.
- Phase interval: 2000 ms.
- Maximum amplitude: 15-20 M.
- Time: 10 minutes.
- Frequency of treatment: Daily for one month.

2. A locally designed treadmill. A modified treadmill has a powerful motor with a different speed control, which ensures a stable, precise and silent performance. Specifications (height: 10 cm, inclination: 0-25%, velocity: 0.1-5 km/h, belt dimensions: 17 x 30 cm, maximum load: 17 kg).

Procedures

All rats in the experimental group received daily treatment either of electric stimulation for group (2), or active exercises on treadmill for group (3). Every rat received 10 minutes of treatment daily for one month. Active electrode was put on the motor point of the quadriceps muscle through the designed window in the cast, the other electrode was put on the femoral nerve over the groin inserted in a direct contact with slight pressure on the motor point of the right quadriceps muscle through the window found in the plaster cast. At the end of the experiment, the animals were sacrificed with an overdose of ether and the quadriceps muscles were dissected out for histological examination. Preparation of tissues was done by using (Jefte) method.. Histological examination were subjected to the morphometric study "the quantitative description of a structure"² Measurement of the muscle fiber diameter was made by means of a micrometer disc that was placed in the ocular disc of the microscope². The disc is usually calibrated as a line divided into 100 units. This calibration is done for standardization of the measurements taken by the ocular micrometer. All the results were tabulated and the mean number of the maximum muscle fiber diameters was calculated.

RESULTS

The difference in size of muscle fiber between the immobilized and the non-immobilized limb in control, electric stimulation and exercise groups was calculated to detect the percentage of muscular atrophy in the right immobilized limb in relation to the left non-immobilized one. The change in muscle fiber size in each of the three groups were compared to determine the effect of electric stimulation and exercise therapy on prevention of muscle atrophy in physiological immobilization

Group 1 (Control):

Inspection of table 1 reveals that number of survived and investigated animals were seven. The MMFD in the right immobilized limb ranged between 11.83 and 18.08 μ with a mean value of $14.62 \pm 2.1\mu$ and SE was 0.24. The MMFD in the left non-immobilized limb ranged between 16.67 and 26.75 μ with a mean value of $21.28 \pm 3.45 \mu$ and SE was 0.33. The difference in MMFD between the right and left side ranged between 4.84 and 8.67 with a mean value of $6.66 \pm 2.50 \mu$ and SE was 0.17

(Table 1). The percentage of atrophy in this group ranged between 15.38 and 42.27% with a mean value of 30.71 ± 8.42 and SE was 0.51. The decrease in muscle fiber size due to muscular atrophy in the right side was statistically significant as $t = 19.54$ and $P < 0.00001$.

Table (1): Maximum fiber diameter of the quadriceps in control group

Animal No.	Right	Left	Differ.	%
1	15	22.42	7.42	33.09
2	13.75	18.75	5.0	26.67
3	18.08	26.75	8.67	32.40
4	16.5	19.5	3.0	15.38
5	14	24.25	10.25	42.27
6	13.19	20.67	7.48	36.18
7	11.83	16.67	4.48	29.02
Mean	14.62	21.28	6.66	30.71
SD	2.10	3.45	2.50	8.42
SE	0.24	0.33	0.17	0.51

$P < 0.0000$

$t = 19.054$

Microscopic examination: by a photomicrograph of a Transverse Section (TS) showed greatest decrease in muscle fiber size in the right immobilized limb compared with the left non-immobilized side in the control group (Fig. 1a and 1b). Fig. 1a shows normal muscle fibers with peripheral nuclei, and fig. 1b shows atrophied muscle fibers with peripheral nuclei in control group (H & E x 200).

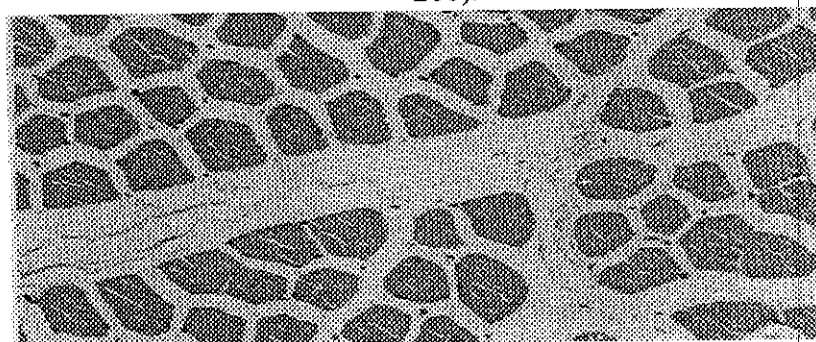


Figure 1a : Normal muscle fiber.

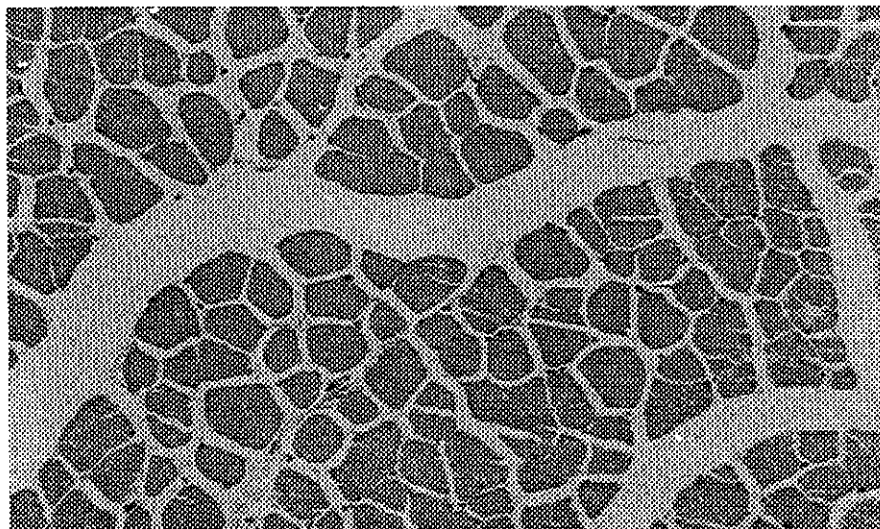


Figure 1b : Atrophied muscle fiber.

Group 2 (Electrical stimulation):

The number of animals who had been survived and investigated in this group were six rats. As shown in table 2 MMFD in the right (immobilized) limb ranged between 18.17 - 24.25 μ with a mean value of $21.98 \pm 2.32 \mu$ and SE 0.42. MMFD in the left (non-immobilized) ranged between 22.0-28.0 μ with a mean value of $26.93 \pm 2.69\mu$ and SE 0.40. The difference in MMFD is 2.17 - 9.17 μ with a mean value of 4.94 ± 2.42 and SE 0.18. The percentage of atrophy ranged between 8.36% - 31.16% with a mean value of 18.08 ± 7.67 and SE 0.61. The decrease in muscle fiber size due to muscular atrophy in the right side was statistically highly significant as $t = 9.28$ and $P < 0.00001$ (Table 2).

Table (2): Maximum muscle fibdiameter of the quadriceps muscle in electricstimulation group.

Animal No.	Right	Left	Differ.	%
1	23.75	25.92	2.17	8.36
2	22.67	27.5	4.83	17.42
3	18.17	22.00	3.83	31.16
4	20.25	29.42	9.17	13.39
5	24.25	28.00	3.75	20.58
6	22.83	28.75	5.92	18.08
Mean	21.98	26.93	4.94	7.67
SD	2.32	2.69	2.42	7.67
SE	0.42	0.40	0.18	0.61

Microscopic examination: by a photomicrograph of a Transverse Section (TS) showed a great decrease in muscle fiber size in the right immobilized limb compared with the left non-immobilized side in the electric stimulation group (Figure 2a and 2b). Fig. 2a shows normal muscle fibers with peripheral nuclei, while fig. 2a shows atrophied muscle fibers with peripheral nuclei (H and E x 200).

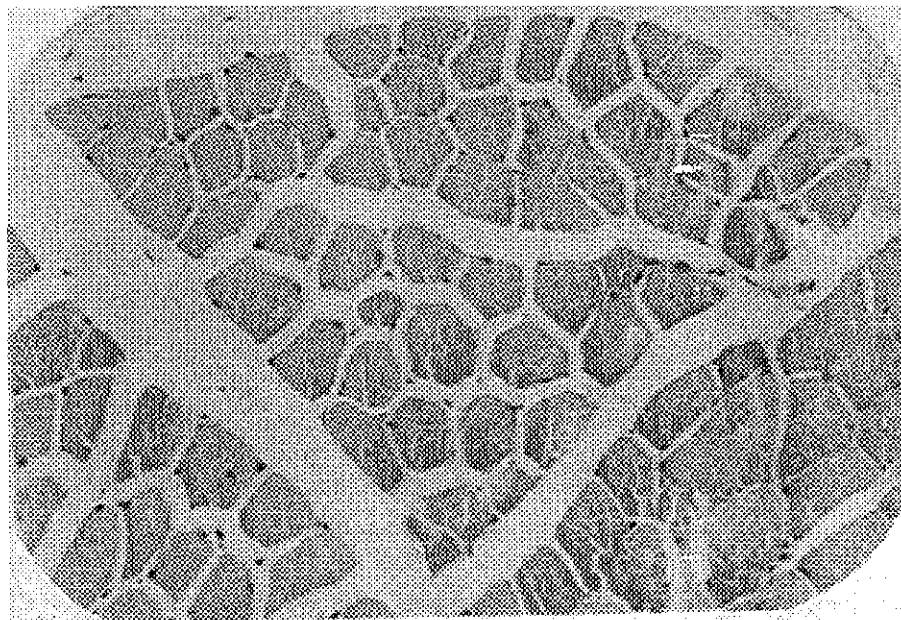


Figure 2a : Normal muscle fiber.

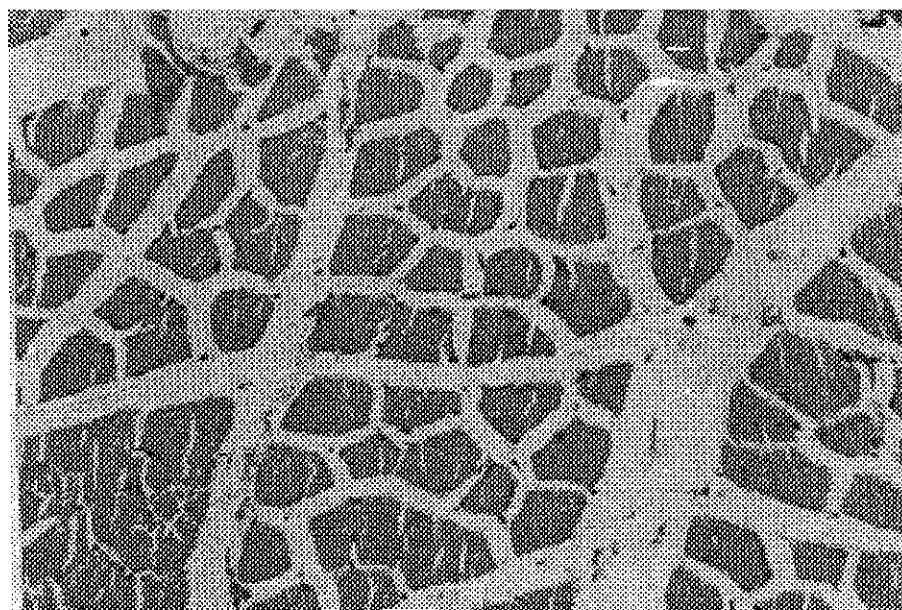


Figure 2b : Atrophied muscle fiber.

Group 3 (active exercises):

The number of animals who had been survived were five rats. As shown in table 3 MMFD in the right (immobilized) limb ranged between 18.05 - 23.56 μ with a mean value of $20.81 \pm 2.07\mu$ and SE 0.35. MMFD in the left (non-immobilized) ranged between 21.25 - 24.00 μ with a mean value of $22.89 \pm 1.18\mu$ and SE 0.34. The difference in MMFD is 0.44 - 3.19 μ with a mean value of 2.07 ± 1.15 and SE 0.08. The percentage of atrophy ranged between 8.82% - 15.00% with a mean value of 9.21 ± 5.28 and SE 0.69. The least decrease in muscle fiber size due to muscular atrophy in the right side was statistically significant as $t = 4.19$ and $P < 0.00001$ (Table 3).

Table (3): Maximum muscle fiber diameter of the quadriceps muscle of the right immobilized limb and left non immobilized limb.

Animal No.	Right	Left	Differ.	%
1	19.81	22.06	2.25	10.20
2	18.06	21.25	3.19	15.00
3	20.75	23.81	3.06	12.86
4	2.97	24.00	0.44	1.82
5	22.89	23.31	1.44	6.17
Mean	20.81	22.89	2.07	9.21
SD	2.07	1.18	1.15	5.28
SE	0.35	0.34	0.08	0.69

Microscopic examination: showed by a photomicrograph of a TS greatest decrease in muscle fiber size in the right immobilized limb compared with the left non-immobilized side in the three groups (Figure 3a and 3b).

Fig. 3a and 3b A photomicrograph of a TS of the normal muscle fiber size (left side), and immobilized muscle fiber size showing atrophied muscle fibers with peripheral nuclei in exercise group (H & E x 200).

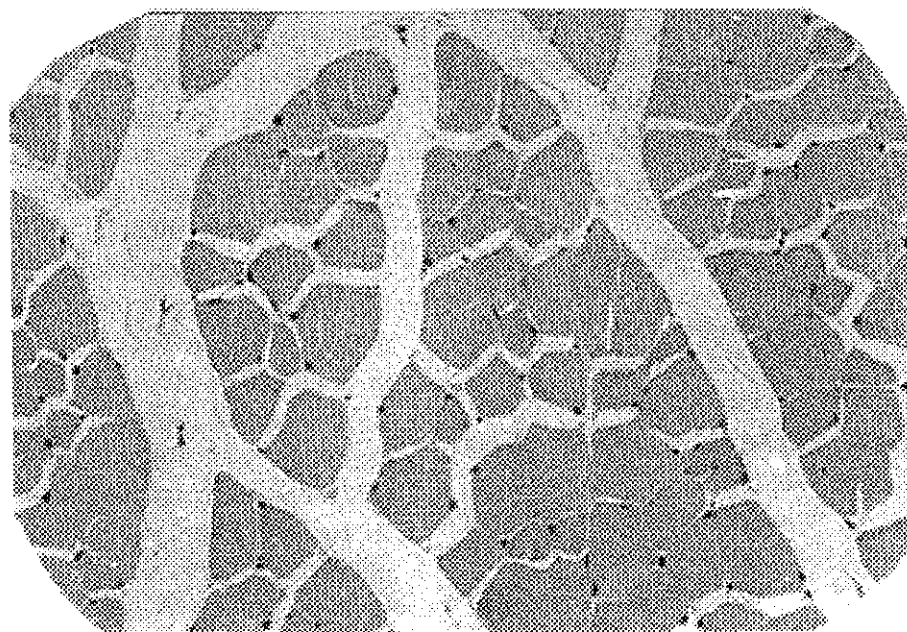


Figure 3a : Atrophied muscle fiber.

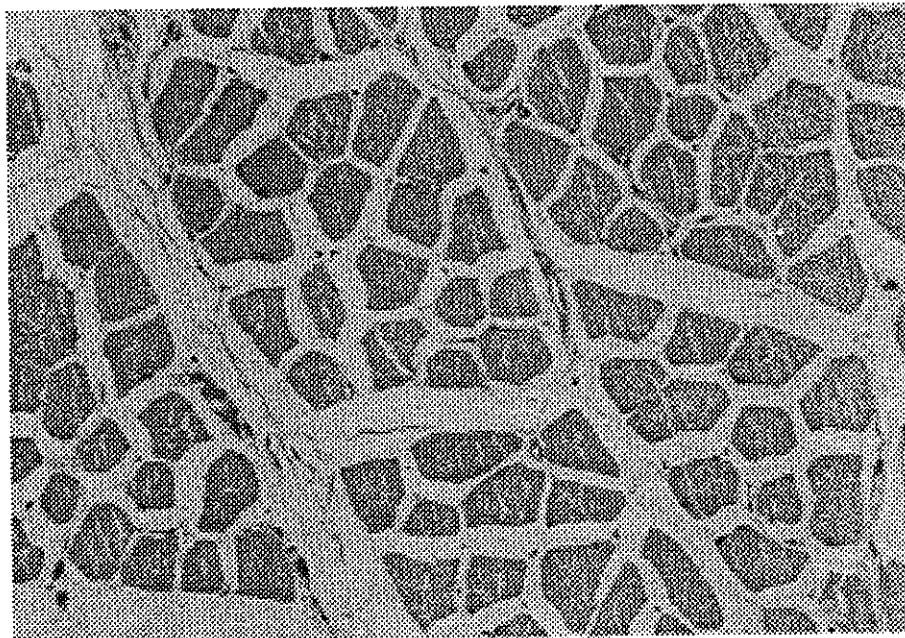


Figure 3b

DISCUSSION

Muscle Fiber Response to Immobilization

The most obvious muscle's sign of immobilization is atrophy. Weible¹⁹ reported that the magnitude of atrophy is different in fast and slow muscle fiber of the dog quadriceps muscle in response to 10 weeks.

Immobilization may cause proliferation of endomysial and perimysial connective tissue relative to control legs, with a significantly greater increase in the immobilized vastus medialis and lateralis muscles compared to rectus femoris muscles.

Thomason et al.¹⁷ showed that very soon after hindlimb unloading, soleus muscles decreased their protein synthetic rate by about 50%, and this rate remained relatively constant up to unloading 7 days.

Babij & Booth³ concluded that while hindlimb unloading resulted in down-regulation of both transcription and translation, the dramatic synthesis rate decrease was primarily due to the down-regulation of translation. Booth and

Thomason¹⁷ reported that the more RNA in the muscle cell, such that α -actin RNA concentration does not limit the synthetic rate. They reported also that regulation does occur at many different levels within the cell.

Effect of Exercise on Muscle Fiber Size

Hypertrophy, as the net result of active exercise, is documented by different research studies. Baldwin et al., and Roy et al.,^{4,14} who performed a series of experiments to define muscle and muscle fiber responses to compensatory hypertrophy. They indicated that the muscle grew proportional to the muscle cross-section or increase in protein. The mechanism of muscle hypertrophy is illustrated by Moritani and Devries¹³. They that this physiological hypertrophy may be attributed to increased in the amount of protein in muscle fiber and an increase in the density of the capillary bed. Significant reduction of calcium transport activity of the overloaded muscle is indicated also by them. The relation

3. Babij, P., and Booth, F.: "Actin and cytochrome CRNAs in atrophied adult rat skeletal muscle". Am.J.Physiol. 254, 1988.
4. Baldwin, K., Valdez, V., Herrick, R., Mackintosh, A., and Roy, R.: "Biomechanical properties of overload fast-twitch skeletal muscle". J. Appl. Physiol. 53:467-472, 1982.
5. Barbary, A.: "A new method for measuring the maximal nuclear diameter by using plastic sheet template of 41-concentric circles (2mm spaced). A modification of Weible outscript inscript circle method". 1995.
6. Cooper, R.: "Alterations during immobilization and regeneration of skeletal muscles in cats". J. Bone Joint Surg. 54-A: 919, 1972.
7. Duchataeu, J., and Hainaut, K.: "Training effect on muscle fatigue in man". Eur. J. Appl. Phys. Med. 53:248-252, 1984.
8. Eisenberg, B., and Gilai, A.: "Structural changes in single muscle fibers after sat a low frequency". J. Ge. Physiol. 74:1, 1979.
9. Eriksson, E., Haggmark, T.: "Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery". Am. J. Sports Med. 7:169, 1979.
10. Gollnick, P., Armstrong, R., Saubert, C., Phiehl, K., and Saltin, B.: "Enzyme activity and fiber composition in skeletal muscle of untrained and trained men". J. Appl. Physiol. 33:312-319, 1979
11. Jaffe, M.: Jaffe method. In Levinson C and Macfate (Eds.): Manual clinical laboratory diagnosis. (7th ed.), PP. 1174, 1969.
12. Levinson, C., and Macfate, C., Heamatoxylin and eosin: Manual clinical laboratory diagnosis, (7th ed.), PP. 1178, 1969.
13. Moritani, T., and Devries, H.: "Neural factors Vs. hypertrophy in the course of muscle strength gained". Am. J. Phys. Med. 58:115, 1979.
14. Roy, R., Medows, I., Balswin, K., and Edgerton, V.: "Functional significance of compensatory overloaded rat fast muscle". J. Appl. Physiol. 52:473-478, 1982.
15. Salmons, S., and Henriksson, J.: "The adaptive response of skeletal muscle to increased use". Muscle and nerve 4:94-105, 1984.
16. Saltin, B., and Gollrick, P.: "Skeletal muscle adaptability: Significance for metabolism and performance. In Peachey, L.D. Handbook of physiology". Bethesda: Am. Physiol. Society, 539-554, 1983.
17. Thomason, D., and Booth, T.: "Atrophy of the soleus muscle by hind limb unweighting". J. Appl. physiol. 68: 1-12, 1991.
18. Vaughen, H., and Goldpink, G.: "Fiber number and fiber size in surgically overloaded muscle". J. Anat. 129:293-304, 1979.
19. Weible, E., and Elias, H.: Introduction to stereological principles. In quantitative methods in morphometry. Weible E. and Elias H (Eds) Berlin, Springer, 1967.
20. Weible, E.: Stereological methods. Vol.1: Practical methods for biological morphometry. New York: Academic Press, 1980.

المختصر العربي

التنبيه الكهربائي مقارنة بالتمارينات في منع ضمور العضلات "دراسة هستولوجية"

أجريت هذه الدراسة لتحديد تأثير التنبيه الكهربائي والتمارينات في منع ضمور العضلي في حالات تثبيت المفصل . هذه الدراسة أجريت على ثلاثون فأراً أبيض من فئران التجارب لمدة شهر كامل وتمت المقارنة بين المجموعات الثلاثة من حيث التغير في حجم الليفة العضلية قبل وبعد التنبيه الكهربائي والتمارينات اليومية وذلك لإيجاد تأثير العلاج بالتنبيه الكهربائي والتمارينات في هذه الحالات وأثبتت نتائج هذه الدراسة أن التمرينات لها تأثير أكبر من التنبيه الكهربائي في منع ضمور العضلات بالمقارنة بالمجموعة المنضبطة .