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EFFECT OF SHORT TERM IMMOBILIZATION ON MECHANICAL CHARACTERISTICS OF SKELETAL MUSCLE IN UROMASTIX

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ABSTRACT:

Gastrocnemius muscles of Uromastix were used to observe the effects of short term (7 days) immobilization in flexed position. Results demonstrated significantly ($P<0.01$) lesser magnitude as well as rate of rise in isometric twitch and tetanic tensions with insignificantly different twitch/tetanus ratio in the immobilized muscles. Both the twitch and tetanus also demonstrated significantly ($P<0.01$) higher twitch half relaxation and twitch/tetanus half relaxation time ratio. While, immobilized muscles showed insignificantly higher twitch contraction, tetanus half relaxation and duration of active state as well as peak twitch duration, than control ones. These changes in the mechanical contraction parameters were not found to be associated with changes in weight, resting, stretched and shortened length of the immobilized muscles. A comparison of present results with previous work performed on skeletal muscles of Uromastix at longer period (20 days) of immobilization has suggested that Uromastix muscles are no prone to a significant immobilization atrophy. However, decreased mechanical response after immobilization irrespective of the period of immobilization in Uromastix muscles is probably due to a depletion in various substrates involved in muscle mechanics.

Introduction

In addition, to the cessation of trophic influence by denervation and cessation of proprioceptive influence by tenotomy, plaster casting on skeletal muscles is also used to find out the degree of muscle atrophy. In this method, the degree of atrophy has been reported to be associated with the disused muscle length and therefore, the effects of immobilization on mechanical properties of skeletal muscles have also been studied by immobilizing the limbs of the experimental animals at shortened, extended and neutral joint positions. Simard *et al* (1982), have demonstrated shorter than normal contraction and half relaxation time in soleus (neutral position), while medial Gastrocnemius (MG) showed no such change at any position of immobilization in rats. They have also reported significantly impaired ability of MG to produce maximal tetanic tension in comparison with its control with no change in the force expressed per whole muscle cross sectional area of MG in either extended or neutral position. Gardiner and Lapointe (1982) have also used rats for immobilization studies. In this study, they fixed the legs of experimental animals in flexed knee and ankle positions in plaster cast which was 70 to 90 degree to their full extension. In these experiments they have demonstrated a faster contraction in the immobilized Soleus and faster half relaxation with lesser twitch durations in the immobilized Gastrocnemius muscles than their respective controls. In addition, lesser twitch and tetanic strength of both the immobilized Soleus and Gastrocnemius was also observed by these authors. In another study, Fitts *et al* (1986) have reported that hind limb immobilization as well as their suspension in rat primarily affects Soleus muscle that showed reduced muscle mass, peak tetanic tension alongwith reduced contraction and half relaxation times in this muscle. Earlier, Witzmann et al (1982) have also demonstrated decreased twitch duration in the immobilized Soleus muscles of rat with reduced contraction and half relaxation times. However, these authors have reported prolonged twitch durations in type IIA and IIB (Extensor Digitorum Longus; EDL) and type IIB (Superficial Vastus Lateralis; SVL) fibers. In addition, they have also reported lesser tetanic tension in Soleus and EDL after immobilization but unaltered tensions in SVL. While the rate of tension development was found by them to decline in Soleus and remained the same in EDL and SVL (fast muscles).

Recently, Azeem and Najam (1992) have also reported significantly lesser tension in 20 days plaster tasted Gastrocnemius (flexed) as well as Sartorius (stretched) muscles of Uromastix during the summer period. Further, more recently Azeem and Fehmeena (1993) have demonstrated differences in the mechanical behaviour of Sartorius, Gastrocnemius, Peroneus Longus and Peroneus Brevis muscles of Uromastix after 20 days of immobilization and attributed these differences to the degree of stretch applied on these muscles during casting periods.

Immobilization studies on human muscles have also demonstrated an increase in both the twitch contraction and half relaxation times of Triceps Surae after two weeks of immobilization (White *et al*, 1984). These changes have been attributed by them to a change in the excitation contraction coupling mechanism. It is interesting that White *et al.* (1984) did not found significant effects on these parameters when these muscles were immobilized for a longer period. In view of these contradictory results and unequivocal effects of immobilization in mammalian skeletal muscles, alongwith differences in the contractile characteristics of the normal skeletal muscles of Uromastix to those of other animals (Azeem and Shaikh, 1987), as well as observed lesser tensions in the Gastrocnemius muscles after longer period of immobilization (Azeem and Najam, 1992), an attempt has been made to observe the effects of short (7 days) period of immobilization on various contraction parameters of the Gastrocnemius muscles of Uromastix.

Materials and Methods

1. Animals:

For immobilization studies, both the male and female *Uromastix hardwickii*, weighing from 300-500 grams were used. These animals were mostly obtained from Thatta district and were placed in large corrugated cartons and large animal cages.

2. Immobilization:

For immobilization purposes Gypsona plaster bandage which was obtained from Smith Kline and French, Laboratories, Pakistan were used. Strips of 25cm x 2.5 cm and 30 cm x 2 cm were cut from these bandages. The 1st strip was used to immobilize the flexed leg (i.e. Knee joint), while the 2nd strip was used to fix ankle joint in stretched position making Gastrocnemius muscle fixed in the flexed position.

3. Dissection:

At the end of the period of immobilization the experimental animals were killed by decapitation and the Gastrocnemius muscles were dissected out as per method of Azeem and Shaikh (1987) alongwith a piece of bone at the point of origin and the ten-don of Achilles at insertion. These two i.e. the piece of bone and tendon facilitated the fixation of muscle in the muscle chamber during experimentation.

During recordings of twitch and tetanus the muscles were kept dipped completely in the normal Reptilian Buffer (Khalil and Messei, 1962) at a constant temperature of 25°C; maintained by thermostatic bath (Karl Kolb). These muscles were stimulated with 50V strength of 5mS duration for both twitch and tetanus (80/Sec.). The twitch and tetanus thus recorded were used for the measurement and calculations of various parameters according to the method described earlier by Shaikh *et al.* (1979a).

Results

The isometric twitch and tetanus records obtained from the control and immobilized muscles during experimentation were used for the calculation of various parameters. According to these results, the tensions obtained from the control and immobilized flexed Gastrocnemius muscles, demonstrated significantly ($P< 0.01$) lesser values in the immobilized muscles (Fig. 1). However, the calculated values of twitch/tetanus ratio were found insignificantly different among the control and immobilized muscles.

The time dependent tension parameters i.e., rate of rise in twitch and tetanus, when compared between control and immobilized (flexed) Gastrocnemius muscles have also demonstrated significant (P <0.01) differences among them (Fig. 2). On the other hand, the time parameters, i.e., twitch contraction and tetanus half relaxation times were found insignificantly different, whereas the twitch half relaxation and twitch/tetanus half relaxation time ratio were significantly (P<0.01) different, being greater in the immobilized muscles (Table 1). Although, the two durations measured from the twitch records have however demonstrated higher values in the immobilized muscles. But these differences for both the peak twitch and active state were statistically insignificant (Table 1).

The results regarding muscle weight, resting, stretched and flexed lengths also demonstrated insignificant differences among control and immobilized muscles (Table 2).

Discussion

Our results regarding the tension generated by the Gastrocnemius (Fig. 1) showed a significantly lower twitch tension by the immobilized muscles, in comparison to their controls. This effect of immobilization has been related earlier (Herbison, 1978; Booth and Seider, 1979b) to the decreased muscle weight as a result of their atrophy. How-ever, in our experiments, no significant differences were observed in weight, stretched, flexed or resting lengths when compared between control and immobilized muscles (Table 2). It means that immobilization for a period of 7 days was unable to alter the muscle dimensions in terms of its length and weight. It is important to note that al-though, tension generation ability of the experimental muscles have been found to decrease significantly after 7 days of immobilization, but there was no change in resting length (lengthat which maximum tension develops) of these muscles. It is therefore possible that immobilization for a period of 7 days has probably affected the tension generation ability by decreasing only the substrates required for the interaction of cross bridges. Booth and Seider (1979a) have also reported a change in the substrates like glycogen, ATP and creatine phosphate in the immobilized muscles. In addition, maintenance of muscle weight and resting length after immobilization has also been reported (Sziklai and Grof, 1982; Azeem and Fehmeena, 1993). Moreover, the insignificant results of the twitch/tetanus ratio indicated that after immobilization the response to a single (twitch) as well as repetitive (tetanus) stimulation were decreased equally, thus the twitch/tetanus ratio remained unaltered. The decreased tension generation ability after immobilization of the Gastrocnemius muscles has also been found to be associated with decreased rate of their generation (Fig. 2). Since, rate of rise in tension is a valid measure of the interaction of cross bridges, therefore a fault in this parameter in both the twitch and tetanus indicated that immobilization is probably resulted not only in a decline in the substrates required for the interaction of cross bridges but also the enzymes involved, that decreased the rate of interaction as well. Decreased enzymatic activities like of myosin ATPase has already been reported by Melichna and Gutmann (1974). These two authors have observed decreased myosin ATPase activity after immobilization in rat skeletal muscles.

As far as the time dependent parameters are concerned 7 days immobilized Gastrocnemius muscles showed a significantly prolonged twitch half relaxation time and a greater twitch/tetanus half relaxation time ratio. The immobilized muscles also showed a prolonged tetanus half relaxation time in comparison to their control ones. But this difference was statistically insignificant. However, the prolongation in twitch and tetanus half relaxation times after immobilization in the flexed Gastrocnemius mussels may be attributed to an increased calcium concentration in the immobilized mussels as also demonstrated by Booth and Giannetta (1973) in rats. We are of the opinion, that probably an increased availability of calcium in the sarcoplasm for a longer period of time in the immobilized Gastrocnemius muscles might have resulted in delayed detachment of cross bridges and an alteration in the sequestration of calcium hack in the sarcoplasmic reticulum. In fact our opinion of increased availability of calcium, and delayed detachment of cross bridges is supported by increased duration of active state and peak duration in twitch (Table 1) respectively, although, not upto the mark statistically. However, an alteration in the sarcoplasmic reticular capacity to sequester the calcium back is also suggested by White *et al* (1984) and Fitts *et al.* (1986) in rat muscles on the basis of prolonged time durations in twitch and tetanus.

Above discussion regarding the short term immobilization in Gastrocnemius muscles and earlier results of long term immobilization (Azeem and Najam, 1992; Azeem and Fehmeena, 1993) suggest that 10-30 days of immobilization in Gastrocnemius muscles of Uromastix in particular and other muscles in general is unable to produce significant atrophy in them. And a change in various mechanical contraction parameters is probably related with a change in the energy producing substrates, sarcoplasmic capacity for calcium manipulation that tends to alter the rate and degree of cross bridge interaction in the immobilized muscles of Uromastix

Table 1

Average values of twitch contraction, twitch and tetanus half relaxation, twitch/tetanus half relaxation time ratio alongwith the durations of peak twitch and active state measured from isometric twitch and tetanus records obtained from control and immobilized flexed Gastrocnemius muscles of *Uromastix hardwickii*. These experiments were performed at 25°C during the month of April.

S.No.	Time (Sec)	Control	Immobilized	P*
Contraction:				
1.	Twitch Contraction	0.020 + 0.001 (6)	0.023 + 0.002 (6)	P > 0.05
Half Relaxation:				
2.	Twitch	0.040 + 0.004 (6)	0.055 + 0.005 (6)	P < 0.01
3.	Tetanus	0.087 + 0.003 (6)	0.101 + 0.008 (6)	P < 0.05
4.	Twitch/Tetanus Ratio	0.460 + 0.040 (6)	0.596 + 0.045 (6)	P < 0.01
Durations:				
5.	Twitch Peak	0.020 + 0.001 (6)	0.023 + 0.002 (6)	P > 0.05
6.	Active State (mS)	21.160 + 2.070 (6)	22.330 + 2.260 (6)	P > 0.05

P* denotes significance level.

Table 2

Average values of muscle weight, stretched, flexed and resting lengths obtained from the control and immobilized (7 days) Gastrocnemius muscles of *Uromastix hardwickii*. These animals were also used for the recording of isometric twitch and tetanus parameters during the summer month of April.

S.No.	Parameters	Control	Immobilized	P*
1.	Muscle Weight (mgs)	399.667 + 35.270 (6)	387.167 + 34.960 (6)	P > 0.05
2.	Muscle Lengths (cms)			
	a) Stretched	2.880 + 0.447 (6)	2.760 + 0.098 (6)	P > 0.05
	b) Flexed	3.260 + 0.012 (6)	3.200 + 0.114 (6)	P > 0.05
	c) Resting	3.083 + 0.133 (6)	3.017 + 0.014 (6)	P > 0.05

P* denotes significance level.

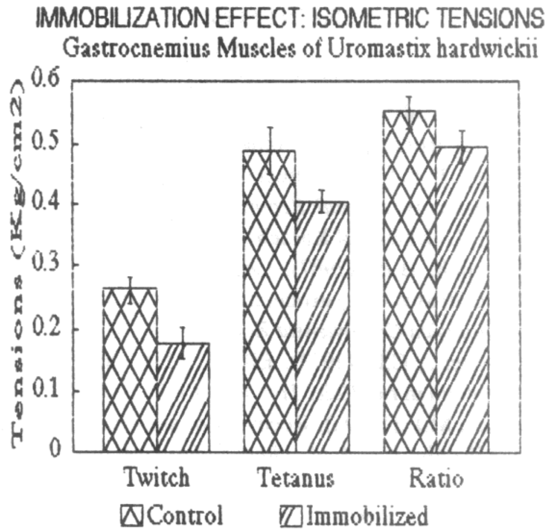

Figure 1: Immobilization effect: isometric tensions.

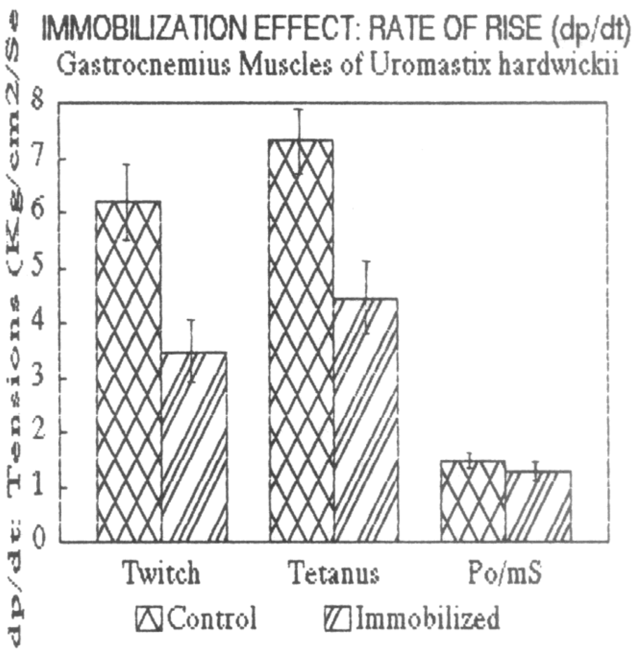

Figure 2: Immobilization effect: rate of rise (dp/dt).

References

Azeem, M.A. and Fehmeena, S. (1993). Effect of plaster cast immobilization and remobilization on skeletal muscles of Uromastix. *2nd Annual Report, N.S.R.D.B. Research Project*, Department of Physiology, University of Karachi, p.l28.

Azeem, M.A. and Najam, U.F. (1992). Effect of plaster cast immobilization on skeletal muscles of Uromastix. *1st Annual Report, N.S.R.D.B. Research Project*, Department of Physiology, University of Karachi, p.4.

Azeem, M.A. and Shaikh, H.A. (1987). Correlation between the anatomical and mechanical characteristics of gastrocnemius and sartorius muscles of *Uromastix hardwickii. Acta Biologica Cracoviensia Zoologia*. **29**: 105-114.

Booth, F.W. and Giannetta, C.L. (1973). Effect of hind limb immobilization up on skeletal muscle calcium in rat. *Calc. Tiss. Res.* **13**(4): 327-330.

Booth, F.W. and Seider, M.J. (1979a). Recovery of skeletal muscle after 3 months of hind limb immobilization. *J. Appl. Physiol. Respirat. Environ. Exercise Physiol.* **47**(2): 435-439.

Booth, F.W. and Seider, MJ. (1979b). Early change in skeletal muscle protein synthesis after limb immobilization of rats. *J. Appl. Physiol. Rasp. Environ. Exec. Physiol.* **47**(5): 974-977.

Fit, R.H., Metzger, J.M., Riley, D.A. and Unworthy, B.R. (1986). Models of disuse: A comparison of hind limb suspension and immobilization, *J. Appl. Physiol.* **60**(6): 1946-1953.

Gardiner, P.F. and Lapointe, M.A. (1982). Daily *in vivo* neuromuscular stimulation effects on immobilized rat hind limb muscles. *J. Appl. Physiol. Resp. Environ. Exercise Physiol*. **53**(4): 960-966.

Herbison, G.J. Jaweed, M.M. and Ditunno, J.F. (1978). Muscle fiber atrophy after cast immobilization in the rat. *Arch. Phys. Med. Rehabil.* **59**(7): 301-305.

Khalil, F. Messeih, A. (1962). Tissue constituents of reptiles in relation to their mode of life: lipid contents. *Comp. Biochem. Physiol.* **6:** 171-174.

Melichna, J. and Gutmann, E. (1974). Stimulation and immobilization effects on con- tractile and Histochemical properties of enervated muscle. *Pflugers Arch. Eur. J. Physiol*. **352**(2): 165-178.

Shaikh, K.N., Shaikh, H.A. Siddiqui, P.Q.R. and Padsha, S.M. (1979a). The temperature dependent contractile properties of normal and denervated muscle. *Nat. Sci.* **1**(1): 33-40.

Simard, C.P., Specter , S.A. and Edgerton, V.R. (1982). Contractile properties of rat hind limb muscles immobilized at different lengths. *Exp. Neurol.* **77**(3): 467-482.

Sziklai, I. and Grof. J. (1982). Effect of immobilization by plastering on the level of various energy metabolites in skeletal muscle *Acta. Physiol. Acad. Sci. Hung.* **59**(3):227-234.

White, M.J., Davies, C.T.M. and Brooks by, P. (1984). The effects of short term immobilization on the contractile properties of the human triceps surae. *Q.J. Exp. Physiol, Cogn. Med. Sci*. **69**(4):685.692.

Witzmann, F.A., Kim, D.H. and Fitts, R.H. (1982). Hind limb immobilization:

Length tension and contractile properties of skeletal muscles. *J. Appl. Physiol. Respir. Environ. Exercise Physiol*, **53**(2): 335-345.