EFFECT OF CAST IMMOBILIZATION ON CONTRACTILE CHARACTERISTICS OF SKELETAL MUSCLES OF UROMASTIX

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Background: Cast immobilized (Imb) mammalian skeletal muscles exhibits reduced tension generation ability with morphometric changes and atrophy. However, controversies still exist with respect to changes in muscle mechanics in different positions of immobilization (IMB). In addition, reptilian muscles have been ignored generally, and for the effects of IMB, specially. This study was carried out to observe the effects of different positions of IMB on contraction parameters of Uromastix skeletal muscles. **Methods:** Left hind limb was Imb in stretched knee and flexed ankle position for 20 days, right being the control (Cont). After 20 days Sartorius (Sar), Gastrocnemius (Gas), Peroneus Longus (PL) and Peroneus Brevis (PB) muscles were isolated to record the isometric twitch and tetani for the measurements of tension, rate of rise of tension (dp/dt) and duration of active state (DAS). Results: After IMB, morphometric parameter does not change significantly in any muscle except the stretched and flexed lengths in Gas. The tensions however, reduced significantly in Imb Sar and Gas which were non-significantly greater in the Imb PL and PB. The dp/dt parameters and DAS reduced in Imb Sar, Gas and PL. It was however, significantly higher in Imb PB. **Conclusion:** Skeletal muscle mechanics differ for different positions of IMB in reptilian muscle model as well. The earlier reported controversies probably indicate differences in IMB induced stretch, dimensions and anatomy of individual muscle of different animal models.

Keywords: Immobilization, Uromastix, Skeletal muscles, Isometric tension parameters.

INTRODUCTION

Plaster cast Imb is well known to produce disuse atrophy. It is found to be associated with morphological[1,] bio-chemical[2], histo-chemical[3], electrical[4] as well as mechanical[5] changes in the experimental muscles. In addition, this atrophy has been reported for its association with changes in both the contractile (myofibrillar) and non-contractile (sarcoplasmic) proteins of the Imb muscles, in different animal models.[6-10] According to Herbison[2,6,7,9] such morphological, ultra-structural[11,12] and morphometric[12-16] changes also occur after IMB. However, magnitude of the above mentioned changes depends on the period[17] and Position of Imb[18] as well as the type of experimental muscles, i.e., fast or slow,[19]. The mechanical changes were found in the tensions[16,20], dp/dt[21] and DAS[22,20] after IMB. However, in some of the studies on reptilian muscle model demonstrated no significant change in the muscle weight and length after IMB, where mechanical parameters affected significantly.[23,24] Although, disuse atrophy has been extensively reported after plaster cast IMB in skeletal muscles, but[25] reported increase in muscle mass on plaster cast Imb in lengthened position. Studies on Imb regarding other shortened and neutral positions are contrary to changes observed in lengthen position.[18] It is important to note that reptilian skeletal muscles have not been used for investigations on the effect of plaster cast IMB and the desert adapted reptile, Uromastix hardwickii, possesses extraordinary muscle strength and also found to be affected by season.[26] Considering the contradiction for the effect of position of IMB[18] and contractile characteristics[27] of reptilian skeletal muscles, present study was designed to observe the effects of different positions of IMB on morphometric parameters and contractile characteristics of skeletal muscles.

MATERIALS AND METHODS

Freshly obtained animals, Uromastix hardwickii, weighing 250–500 gm were used for the experiments according to the standards of International Animal Ethics Committee. Prior to the application of plaster cast to the left hind limb the animals were anaesthetized by injecting 1 ml 2% Xylocaine in the gluteal muscles.[28,29] Whole of the left hind limb from hip joint till phalanges was then plaster casted keeping the knee in stretched and ankle in flexed positions thus, immobilizing Gas in stretched, Sar in neutral and PL and PB in highly stretched positions. The right hind limb was used as Cont. At the end of 20 days of immobilization, the isolated Imb and control muscles were kept in reptilian buffer solution at 25 ºC.[27,30] Isometric twitch and tetanus were then recorded on oscillograph (Harvard apparatus, U.K.) from both the Imb and Cont muscles. Later, these records were used for the measurement of isometric tensions, dp/dt and DAS.[27]

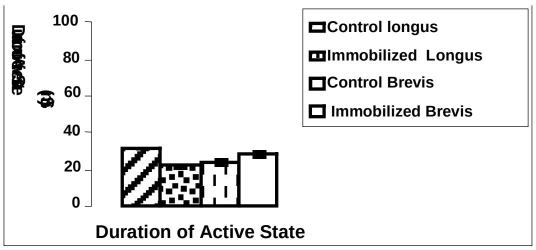
RESULTS

A. EFFECT OF IMMOBILIZATION ON PERONEUS LONGUS AND PERONEUS BREVIS (HIGHLY STRETCHED POSITION):

i) Isometric Tensions:

The average values of isometric twitch and tetanus (Fig.-1a) obtained from the Cont and Imb PL muscles, showed non-significantly (p>0.05) higher values in the Imb ones. However, average values of these parameters obtained from the Cont and Imb PB demonstrated higher values of twitch in the Imb muscles. In case of tetanic tension, IMB has reduced its values. However, these changes were statistically non-significant (p>0.05).

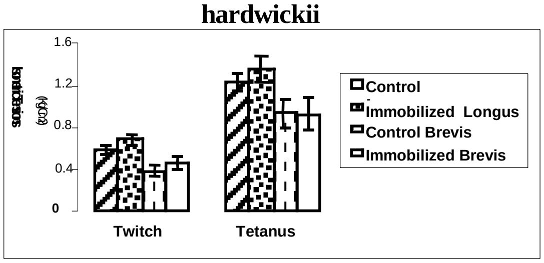
Fig.-1a Comparison of Isometric Tensions Between 20 Days Immobilized & Control Peroneus Longus & Peroneus Brevis Muscles of Uromastix hardwickii



ii) Duration of active state (DAS):

The average values of the DAS obtained from the PL muscles, showed significant (p<0.0005) reduction in this parameter, after IMB, compared to its Cont (Fig.-1b). It showed significantly (p<0.025) higher values in the Imb PB muscles.

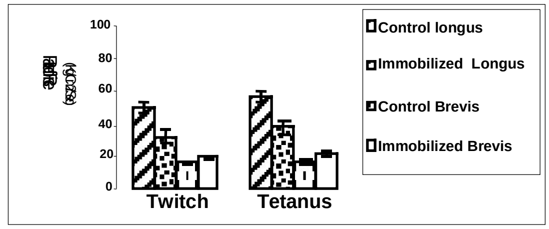
Fig.-1b: Comparison of Active State Duration Between 20 Days Immobilized Longus & Peroneus Brevis Muscles of Uromastix & Control Peroneus hardwickii



iii) Rate Of Rise Of Tension (dp/dt):

The average values of dp/dt in twitch and tetanus have been presented in (Fig.-1c). The PL muscles demonstrated significantly lesser values of these parameters in the Imb muscles, being (p<0.005) and (p<0.0005) respectively. However, the average values of these parameters demonstrated higher values in Imb PB and this difference between the Cont and Imb muscles was significant (p<0.01) for dp/dt twitch and (p<0.025) for dp/dt tetanus.

Fig.-1c Comparison of Rate of Rise in Twitch & Tetanus Between 20 Days Immobilized & Control Peroneus Longus & Peroneus Brevis Muscles of Uromastix hardwickii.

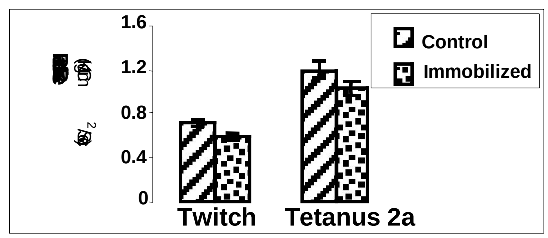


B. EFFECT OF IMMOBILIZATION ON GASTROCNEMIUS (MODERATELY STRETCHED POSITION)

i) Isometric Tension:

The average values of isometric twitch and tetanus (Fig.-2a) obtained from the Cont and Imb Gas muscles, demonstrated lesser average values in Imb muscles in comparison to the Cont ones. This difference between the Cont and Imb muscles was significant (p<0.005 and p<0.05 respectively).

Fig.-2a: Comparison of Isometric Tensions Between 20 Days Immobilized & Control Gastrocnemius Muscles of Uromastix hardwickii



ii) Duration Of Active State (DAS):

The average values of the DAS obtained from Cont and Imb Gas muscles (Fig.-2b) showed non-significantly (p>0.05) lesser values in the Imb muscles when compared to their Cont ones.

iii) Rate Of Rise Of Tension (dp/dt):

The average values of dp/dt in twitch and tetanus obtained from Cont and Imb Gas muscles demonstrated non-significantly (p>0.05) lesser values of these parameters in the Imb ones (Fig.-2c).

Fig.-2b: Comparison of Active State Duration Between 20 Days Immobilized & Control Gastrocnemius Muscles of Uromastix hardwickii

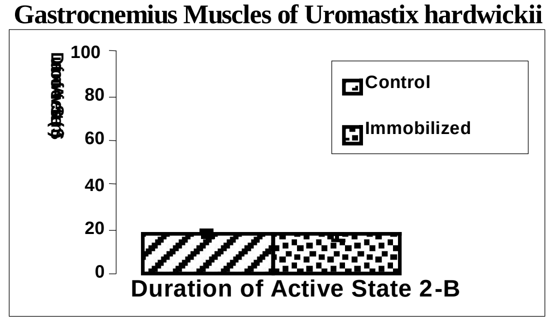


Fig.-2c:Comparison of Rate of Rise in Twitch & Tetanus Between 20 Days Immobilized & Control Gastrocnemius Muscles of Uromastix hardwickii.

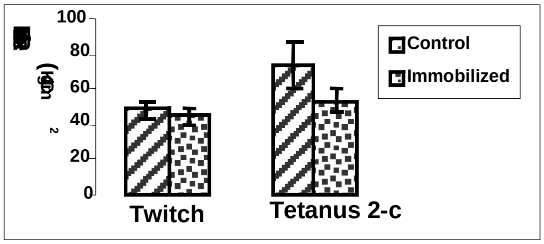
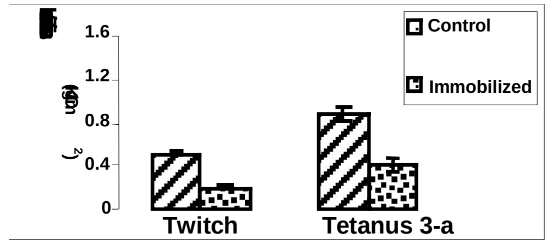


Fig.-3a: Comparison of Isometric Tensions Between 20 Days Immobilized & Control Sartorius Muscles of Uromastix hardwickii



C. EFFECT OF IMMOBILIZATION ON SARTORIUS (NEUTRAL POSITION).

i) Isometric Tensions:

The average values of isometric twitch and tetanus (Fig.-3a) obtained from the Cont and Imb Sar muscles were found significantly ($p<0.0005$) lesser in the Imb muscles when compared with their Cont.

ii) Duration Of Active State (DAS):

The average values of DAS obtained from the Cont and Imb Sar muscles have been presented in (Fig.-3b). These average values have been found to be reduced significantly ($p<0.0005$) on IMB.

iii) Rate Of Rise Of Tension (dp/dt):

The average values of dp/dt in twitch and tetanus (Fig.-3c) obtained from the Cont and Imb Sar demonstrated significantly ($p<0.0005$) lesser values in the Imb muscles.

Fig.-3b Comparison OF Active State Duration Between 20 Days Immobilized & Control Sartorius Muscles of Uromastix hardwickii

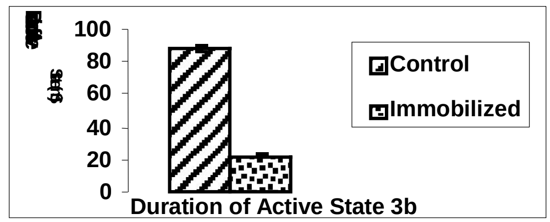


Fig.-3c: Comparison of Rate of Rise in Twitch & Tetanus Between 20 Days Immobilized & Control Sartorius Muscles of Uromastix hardwickii.

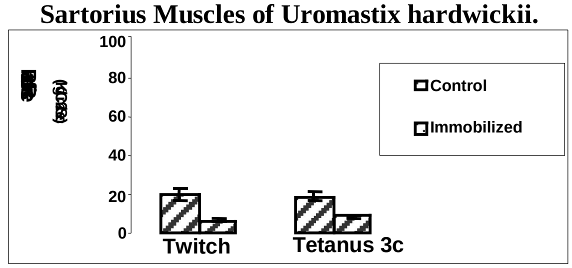


Table-1: Comparison of various morphometric parameters obtained from control and 20 days immobilized Gastrocnemius, Peroneous Longus, Peroneous Brevis and Sartorius muscles of Uromastix hardwickii. Mean±SE (number of muscles used)

Muscle Type	Stretched Length	Flexed Length	Resting Length	Muscle Weight
1. Gastrocnemius				
a) Control	2.940±0.049 (15)	2.707±0.046 (15)	3.327±0.033 (15)	897.973±44.915 (15)
b) Immobilized*	3.067±0.045 (15)	2.833±0.043 (15)	3.293±0.038 (15)	876.920±46.131 (15)
	$p<0.05$	$p<0.025$	$p>0.05$	$p>0.05$
2. Peroneus Longus				
a) Control	2.606±0.031 (16)	2.419±0.029 (16)	2.4±0.033 (16)	247.800±14.592 (16)
b) Immobilized**	2.654±0.046 (13)	2.462±0.047 (13)	2.423±0.041 (13)	226.415±15.959 (13)
	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$
3. Peroneus Brevis				
a) Control	2.921±0.039 (14)	2.714±0.036 (14)	2.893±0.056 (14)	188.579±12.556 (14)
b) Immobilized**	2.946±0.033 (13)	2.738±0.035 (13)	2.908±0.054 (13)	172.562±10.656 (13)
	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$
4. Sartorius				
a) Control	4.086±0.068 (14)	3.636±0.056 (14)	4.057±0.063 (14)	1003.45±50.016 (14)
b) Immobilized***	4.131±0.064 (13)	3.623±0.051 (13)	3.923±0.059 (13)	1049.53±73.043 (13)
	$p>0.05$	$p>0.05$	$p>0.05$	$p>0.05$

*Moderately Stretched, **Highly Stretched, ***Neutral

DISCUSSION

According to above results, the Gas muscles that were Imb in moderately stretched position, did not show significant change in their morphometric parameters (Table-1) except their stretched and flexed lengths increased significantly which is not evident in Sar, due to its neutral position maintained during IMB. In our opinion, significantly higher stretched and flexed lengths observed in Imb Gas in comparison with their Cont, is because of a compensatory reduction in the operating range of Cont that is reflected as an increased stretched and flexed lengths of Imb Gas muscles. It means that there was no longitudinal growth in muscle fibres of moderately stretched Imb Gas muscles as reported earlier[31] with the concept of addition of sarcomeres.

However, reduced tension generation ability in Imb Sar and Gas muscles in the absence of significant changes in muscle weight and resting length indicate a probable reduction in the available energy substrate.[32,33] In this connection, Booth and Seider[5] demonstrated significant reduction in the concentration of Adenosine triphosphate along with Glycogen contents after 90 days of Imb in rat skeletal muscles. Stanish[34] also demonstrated reduction in the ATPase activity and Glycogen content in human Vastus lateralis muscles after 6 weeks of Imb. According to the above references it is expected that there was a significant reduction in energy substrates, i.e., ATP content causing reduced tension generation ability in both the Sar and Gas muscles in the absence of changes in their weight after IMB. Another possibility for reduced tension generation may be referred to their decreased ability to utilize energy during force generation as reported by[35] in Imb rat hind limb Soleus and Gas muscles. It is to be noted that rate of reaction during contraction is basically determined by the activity of myosin ATPase[36] and number of activated cross bridges in myofilament that determines the degree of tension generation. It is therefore the reason that dp/dt (Fig.-3c, 2c) were reduced in our experimental Sar and Gas after IMB. It is further supported by the reduction in the DAS in both of these muscles, though non-significant in Gas, which is due to its moderately stretched position that probably resisted the effects of IMB.

In-addition, the lesser values of DAS demonstrated by Imb Gas ($p>0.05$) and Sar ($p<0.0005$) along with a decline in their dp/dt twitch and tetanus indicate a probable alteration in the function of sarcoplasmic reticular structure of these muscles probably leading to a decreased release of calcium from sarcoplasmic reticulum. Azeem and Shaikh[37,27] have associated twitch peak and DAS with the presence of free ionic calcium as well as prolong interaction of calcium with cross bridges in Uromastix Sar and Gas muscles. In our experiments, reduction in both the tension generation and DAS means that the calcium dependent tension generation ability both in terms of magnitude and rate of its development is reduced in these muscles after IMB. However, the non-significant reduction in the above mentioned parameters obtained for Gas reflect its moderately stretched position, resisting the effects of IMB.

PL and PB muscles did not undergo significant change in any of their morphometric parameters (Table-1) in spite of their highly stretched position maintained during IMB. Similarly, both the Imb PL and PB muscles demonstrated non-significantly higher values of isometric tensions compared to their Controls. It means PL and PB muscles, that were Imb in highly stretched position have failed to suppress the effects of IMB. On contrary to our results for PL and PB, increase in tension generation ability of rat skeletal muscles Imb in lengthened position had been reported earlier[38,39]. Further, Stretch related increase in tension generation had been claimed to be associated with an increase in muscle weight, fibre length, fibre diameter and sarcomere number.[40,31] While, in the present study non-significant rise in tension generation, in Imb PL and PB muscles means that either some intrinsic changes in the Imb PL and PB were responsible to resist the usual effects of IMB or a comparatively lesser cross sectional area/volume of these muscles than Gas and Sar, did not allow addition of sarcomeres for longitudinal or transverse growth. [31] However, Imb PB muscles demonstrated significant increase in dp/dt and DAS. In this connection, Dowsan[41,42] suggested that increased dp/dt is limited by the rate of calcium release while, the rate of calcium re-uptake controls the rate of decline in tension generation ability of the muscles. In another study[43] have demonstrated that twitch duration and dp/dt in twitch and tetanic tensions are related to alterations in sarcoplasmic reticular function. Therefore, we are of the opinion that, IMB induced stretch in our experimental PB muscles has resulted in some changes at sarcoplasmic level leading to increased rate of calcium release for greater interaction with cross bridges and its slow re-uptake in SR. Thus, increasing its tension generation along with its rate of development as well as the DAS. On the other hand, the PL muscles that were also Imb in highly stretched position, demonstrated significantly lesser values of dp/dt and DAS compared to the Cont muscles. In our opinion, this difference between the Imb PL and PB muscles in spite of being Imb in highly stretched position the different degree of stretch was induced on their fibres and tendons. Thus, affecting primarily the tendon part of PL while, both the tendon and the muscle fibres were affected in PB. On the basis of above discussion regarding the effects of IMB on the contraction parameters of Uromastix skeletal muscles, we conclude that, IMB reported in animals other than uromastix exhibits reduction in their tension generation ability associated with disuse atrophy. However, the skeletal muscles of the reptile uromastix resisted the disuse atrophy but a decline in their tension generation ability is probably associated with decreased energy substrates as discussed earlier. Further stretch related rise in tension generation ability depends upon the degree of stretch offered during IMB, affecting the muscle fibre or tendon of insertion. It is also suggested that the controversial results regarding position of IMB depends upon not only on the type of skeletal muscle but also on the animal model.[3]

REFRENCES

1. Appell HJ. Morphology of immob ilized skeletal muscle and the effects of a preimmobiliza tion and postimmobilization training program. Int J Sports Med 1986;79(1):6-12.
2. Booth FW, Seidar MJ. Early cha nges in skeletal muscle protein synthesis after limb i mmobilization of rats. J Appl Physiol Respirat Environ Exercise Physiol.1979b;47:974-7.
3. Pattullo MC, Cotter MA, Camero n NE, Barry JA. Effects of lengthened immobilization on function al and histochemical properties of rabbit tibialis anterior muscle. Exp Physiol (1992);77(3):433-42.
4. Dudley GA, Duvoisin MR, Adams GR, Meyer RA, Blew AH, Buchanan P. Adaptations to uni lateral lower limb suspension in humans. Aviate Space Environ. Med 1992;63(8):678-83.
5. Petit J, Gioux M. Properties o f motor units after immobilization of cat peroneus longus muscle. J Appl Physiol. 1993;74(3):1131-9.
6. Isfort RJ, Wang F, Greis KD, Sun Y, Keough TW, Bodine SC, Anderson NL. Proteomic analysis of rat soleus and tib ialis anterior muscle following immo bilization. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;769(2):323-32.
7. Herbison G J, Jaweed MM, Ditunno JF. Muscle fiber atrophy after cast immobilization in the rats. Arch Phys Med Rehabil. 1978;59(7):301-5.
8. Kauhanen S, Leivo I, Michelsso n JE. Early muscle changes after immobilization an experi mental study on muscle damage. Clin Orthop. 1993;(297):44-50.
9. Miles MP, Clarkson PM, Bean M, Ambach K, Mulroy J, Vincent K. Muscle function at the wrist following 9 days of immobilization and suspension. Med Sci Sports Exerc. 1994;26(5):615-23.
10. St. Amand J, Okamura K, Matsum oto K, Shimizu S, Sogaw Y. Characterization of control and immobilized skeletal muscle: an overview from genet ic engineering. Faseb J. 2001;15(3):684-92.
11. Leivo I, Kauhanen S, Micheisson JE. Abnormal mitochondria and sarcoplasmic changes in rabbit skeletal muscle induced by immobilization. Apmis. 1998;106(12):1113-23.
12. Smith HK, Maxwell L, Martyn JA , Bass JJ. Nuclear DNA fragmentation and morphological alt erations in adult rabbit skeletal muscle after short -term immobilization. Cell Tiss ue Res. 2000;302(2):235-41.
13. Heslinga JW, Huijing PA. Effec ts of short length immobilization of medial gastr ocnemius muscle of growing young adult rats. Eur J Morphol. 1992;30(4):257-73.
14. Heslinga JW, Huijing PA. Muscl e length-force characteristics in relation to muscle architec ture; a bilateral study of gastrocnemius medialis muscles of unilaterally immobilized rats. Eur J Appl Physiol. 1993;66(4):289-98.
15. Veldhuizen JW, Verstappen FT, Vroemen JP, Kuipers H, Greep JM. Functional and morph ological adaptations following 4 weeks of knee immo bilization. Int J Sports Med. 1993;14(5):283-7.
16. Ansved T. Effect of immobiliza tion on rat soleus muscle in relation to age. Acta Physiol Scand. 1995;154(3):291-302.
17. McDonald KS, Blaser CA, Fitts RH. Force velocity and power characteristics of rat soleus muscle fibres after hind limb suspension. J Appl Physiol. 1994;77(4):1609-16.
18. Karpakka J, Virtanen P, Vaanan en K, Orava S, Takala TE. Collagen synthesis in rat skel etal muscle during immobilization and re -mobilization. J Appl Physiol 1991;170:389-96.
19. Boyes G, Johnston I. Muscle fi bre composition of rat vastus intermedius following immobili zation at different lengths. Pflugers archer J physiol. 1979;381(3):195-200.
20. Seki K, Taniguchi Y, Narusawa M. Alterations in contractile properties of human skeletal m uscle induced by joint immobilization. J Physiol. 2001;530(3):521-32.
21. Duchateau J, Hainaut K. Effect s of immobilization on the contractile properties, recruitment and firing rates of huma n motor units. Lab of Biology Univ of Brussels. 1990;422:55-65.
22. Simard CP, Spector SA, Edgerto n VR. Contractile properties of rat hind limb muscle immobi lized at different lengths. Exp Neural. 1982;77(3):467-82.
23. Azeem MA, Najam UF. Effects of plaster cast immobilization on skeletal muscles of Uromast ix. Ist Annual report, N.S.R.D.B. research project, D epartment of physiology, University of Karachi. 1991;p-4.
24. Azeem MA, Irfan T, Fehmina S, Arifa S, Najam UF. Effects of short term immobilization on mechanical characteristics of skeletal muscle in Uromastix. Pak J Pharm Sci. 1993;6(2):9-17.
25. Tabary JC, Tabary C, Tardieu G, Goldspink G. Physiological and structural changes in the cat soleus mu scles due to immobilization at different lengths by plaster casts. J Phys iol (Lond). 1972;224:231-44.
26. Azeem MA, Shaikh HA. Effect o f Season on length tension relation of gastrocnemius musc le of Uromastix hardwickii. Pak J Physiol 2006;2(1):17-21.
27. Azeem MA. Seasonal and temperature var iations in the mechanical contraction, strength duration and length tension properties of skeletal muscles of Uromastix. Ph. D. Thesis Dept of Physiology University of Karachi, Pakistan. 1992.
28. Arifa S. Effect of season on isometric contraction properties of the tenotomized gastrocnemius muscles of Uromastix hardwickii. Msc. Thesis Univ of Karachi, Pakistan. 1990.
29. Shahina N. Effect of season on isometric contraction properties of the chronically denervated gastrocnemius muscles of Uromastix hardwickii. Msc. Thesis University of Karachi, Pakistan. 1990.
30. Khalil F, Messeih G. Water con tent of desert adapted reptile and mammals. J Exp Zool. 1954;125:407-17.
31. Williams PE, Goldspink G. Chan ges in the sarcomere length and physiological properties in immobilized muscles. J Anat. 1978;127(3):459-68.
32. Didyk RB, Anton EE, Robinson KA, Menick DR, Buse MG. Effect of immobilization on gl ucose transporter expression in rat hind limb muscles. Metabolism. 1994;43(11):1389-94.
33. Op‘t Eijnde B, Urso B, Richter EA, Greenhaff PL, Hespel P. Effect of oral creatine supple mentation on human muscle GLUT4 protein content after im mobilization. Diabetes. 2001;50(1):8-23.
34. Stanish WD, Valiant GA, Bonen A, Belcastro AN. The effects of immobilization and of elect rical stimulation on muscle glycogen and myofibrillar ATPa se. Can J Appl Sport Sci. 1982;7(4):267-71.
35. Krieger DA, Tate CA, McMillan -Wood J, Booth FW. Population of rat skeletal mus cle mitochondria after exercise and immobilization. J Appl Physiol Respirat Environ Exercise Physiol. 1980;48:23-8.
36. Szoor A, Rapcsak M, Hallasi G. Effects of plaster cast immobilization on the contract ile properties of rat skeletal muscles. Acta Biol. 1981;32(2):129-30.
37. Azeem MA, Shaikh HA. Seasonal variations in the contractile behavior of gastrocnemius muscles o f uromastix hardwickii. Acta Physiologica 1991;77(2):59-68.

38. Goldberg AL. Work induced growth of skeletal muscles in normal and hypophysectomised rats. A J Physiol. 1967;213:1193-8.
39. Mackova E, Hnik P. Compensatory muscle hypertrophy induced by tenotomy of synergists is not true working hypertrophy. Physiologia Bohemoslovenica 1973;22:43-9.
40. Crawford GNC. The growth of striated muscle immobilized in extension. J Anat 1973;114:165-83.
41. Dawson MJ, Gadian DG, Wilkie DR. Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorus nuclear magnetic resonance. J Physiol (London). 1980;299:465-84.
42. Fitts RH, Courtright JB, Kim DH, Witzmann FA. Muscle fatigue with prolonged exercise: Contractile and biochemical alterations. Am J Physiol 1982;242:C65-71.
43. Witzmann FA, Kim DH, Fitts RH. Hind limb immobilization: Length tension and contractile properties of skeletal muscles. J Appl Physiol Respir Environ Exercise Physiol 1982;53(2):335-45.

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