EFFECT OF SEASON ON LENGTH-TENSION RELATION OF GASTROCNEMIUS MUSCLE OF UROMASTIX HARDWICKII

Azeem MA, Shaikh HA*.

Neuromuscular Unit, Department of Physiology, University of Karachi, Karachi, Pakistan. *Department of Physiology, Ummul-Qura University, Mecca, Saudi Arabia.

Background: Seasonal variations in the biological activities of animals are commonly reported in literature. However, these variations are not studied yet in correlation with the structure and function of skeletal muscles in general and their mechanics in particular. **Methods:** This study was conducted on skeletal muscles of a reptile, Uromastix, to determine the effect of season on the shape of length-tension curves, active tension, passive tension and tension equilibrium length (TEL). **Results:** Result demonstrates that active tension obtained from gastrocnemius muscle was found to increase significantly from the winter (December) to peak summer month (June), which showed a significant fall till the second winter (December). The passive tension was also found to increase significantly ($P<0.0005$) from winter (December) to peak summer (June) which also decreased significantly till the second winter (December). Change in both the active and passive tensions has resulted in a rise in the average values of tension equilibrium length from the winter (December) to peak summer (June) and fall till second winter (December). **Conclusion:** It is concluded that length-tension parameters exhibit variations between different seasons and reflects a dominancy of contractile elements towards summer and elastic elements towards winter in the gastrocnemius muscles of Uromastix.

Key Words: Contractile, Elastic, Length-Tension relation, Season, Tension Equilibrium length, Resting Length, Uromastix, Gastrocnemius.

INTRODUCTION

Seasonal variations in the biological activity of various classes of animals had been reported by many workers[1,-6] The reptile Uromastix has also been used for some other studies, i.e., histochemistry of lingual salivary gland and contractile proteins of skeletal muscles.[7, 8] Variation in testis size[9], changes in body weight[10] and changes in plasma electrolyte concentration[11] have also been reported.

On the other hand, the mechanical behavior of skeletal muscle is largely discussed in literature in terms of length-tension relation that provides interpretation of sliding filament theory, tested by measuring the tension of muscle fibre at different sarcomere length[12]. In addition, length-tension relationship of whole skeletal muscle has been used for the explanation of its contractile and elastic state. Common length-tension parameters used for such explanation are Active tension, Passive tension, Initial length and resting length of skeletal muscle. However, Changes in mechanical characteristics of skeletal muscles of reptiles and seasonal changes in terms of length tension relation parameters are not available in literature.

In this connection, an earlier study[13] on length tension relation of the skeletal muscles of reptile Uromastix hardwickii, the muscle length, at which both the active and passive tensions are equal, has been termed as Tension Equilibrium Length (TEL) [13]. In the present study, the gastrocnemii of the reptile Uromastix hardwickii were used to obtain a series of length-tension curves during various periods of a seasonal cycle. The effect of season was observed on the basis of changes in the shape of length-tension curves, values of active and passive tensions and tension equilibrium length throughout the year to ascertain the length-tension characteristics of skeletal muscle of this reptile Uromastix hardwickii.

MATERIAL AND METHODS

Reptilian Buffer Solution

The composition of this reptilian buffer solution was same as described by[14] NaCl, 100mM; KCl, 3.8mM; $CaCl_2$, 1.8mM; KH_2PO_4, 1.2mM; Na_2HPO_4, 5.8mM. All the chemicals used in the present study were obtained from E.Merck.

Equipments

a) Universal Oscillograph, (Cat-No 50-8622)
b) Isometric force transducer, (Cat-No 50-7913)
c) Macromanipulator (C.F. Palmer, London)
d) Dual impedance research stimulator, (Harvard Cat No: 50-7459) (50v, 0.5mS, 1Hz)
e) Thermostatic circulator bath model RMT6 (Cat No: 232-030)
f) Muscle chamber.

Muscle Isolation, Fixation and recording from gastrocnemii

Both the sexes of adult reptile Uromastix hardwickii (100-150gm) were used in all the experiments reported here. The animals were killed by decapitation that meets the ethical standard. Then, gastrocnemii of both limbs were dissected out immediately with knee joint and Achilles tendon. The dissected muscles were immediately transferred into muscle chamber, containing reptilian buffer. Its temperature (20^0C) was regulated through a circulator thermostatic bath.

Proximal end of muscle having knee joint was fixed to a pin present in the muscle chamber. Its distal end having Achilles tendon was attached through a hook and thread passing through a pulley for attachment with Isometric Force Transducer.

For the stimulation of muscle, a pair of stimulating silver chloride electrode was placed beneath the muscle. The stimulating electrode was connected to D.C stimulator.

For recording purpose the muscle was first attached at its flaccid length, where minimum tension was recorded on twitch stimulation (50V, 5msec, 1pulse/sec). The initial flaccid length was measured with the help of a divider & ruler, leaving the tendons of origin & insertion. Stretch was then applied to the experimental muscle through macromanipulator. At each step, when muscle was stretched by 1mm, it was also stimulated for the recording of isometric twitch tensions, till a length at which active tension declines. The passive tension was measured as pen deflections at each of the above steps before stimulation.

Calculation of Tension

Isometric tensions were measured and calculated by using the following formula.

$$\text{Tension (Kg/cm}^2) = \frac{\text{Tension (gm)} * \text{muscle length (cm)}}{\text{Muscle weight (mg)}}$$

Whereas tension in gm was calculated as

$$\text{Tension (gm)} = \frac{10 * Y}{X}$$

Where, X = pen deflection (mm) with a known weight of 10gm hanging on transducer's leaf. Y = Maximum pen deflection (mm) on twitch development.

Construction of length-tension curve:

In all the experiments, the length-tension curves were constructed by plotting active and passive tensions against muscle length. Individual length-tension curves were then used to calculate the tension equilibrium length.

Method for the measurement of Tension Equilibrium Length (TEL):

A perpendicular was drawn (Fig. 1) from the point 'a' where active and passive tension curves crossed each other, till it intercepted the x-axis at point 'b'. The muscle length read at point 'b' on the x-axis was taken as TEL, at which the active and passive tensions were equal. While the length at which maximum active tension was obtained has been taken as resting length (Lo).

Statistical analysis:

Statistical analysis included the calculations of Standard deviation and standard error for the analysis of data. Statistical comparison was performed by using student's t – test (two tailed) at the level of significance, 0.05.

RESULTS

Maximum active tension obtained from gastrocnemius muscle was found to increase significantly by about 3.2 folds from the winter (December) to peak summer month (June). Later, it showed a significant fall that continued till the second winter (December) as shown in Table.1. The passive tension was also found to increase significantly ($P<0.0005$) from winter (December) and continued to rise till the peak summer (June). Later, passive tension showed a gradual but significant fall till the second winter (December) as shown in Table.1.

A non-significant ($P>0.05$) rise was observed in the average values of TEL from the winter (December) to peak summer (June). Later, it decreased till second winter (December) as shown in Table.2.

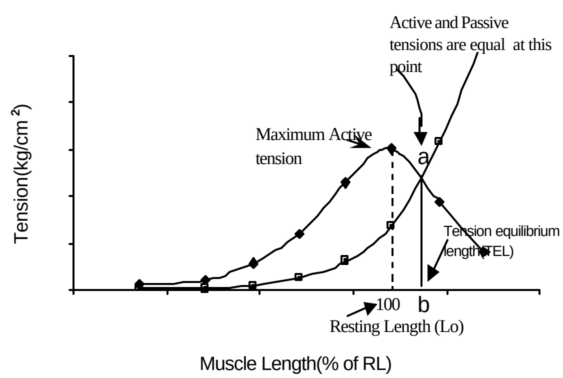

Fig. 1. Method for the measurement of Tension Equilibrium Length (TEL)

DISCUSSION

Length-tension curves obtained from the gastrocnemius muscles of uromastix during various periods of a seasonal cycle (Fig. 2) have demonstrated a marked effect on their shapes by affecting their magnitude. The active twitch tension

Table-1: Effect of Season on the isometric active and passive tensions.

The values were calculated from their respective length tension curves obtained from the gastrocnemius muscles, during the period ranging from December to December

Season		Isometric Tensions at resting length (kg/cm^2) Mean ±SE	
MONTH	DAYS	ACTIVE TENSIONS	PASSIVE TENSIONS
December	13	0.088±0.012 (n=16)	0.064±0.009 (n=16)
January	43	0.228±0.017 (n=16)	0.210±0.027 (n=16)
February	82	0.142±0.010 (n=8)	0.074±0.013 (n=8)
March	105	0.244±0.021 (n=13)	0.184±0.016 (n=13)
April	143	0.149±0.016 (n=8)	0.091±0.015 (n=8)
June	195	0.287±0.050 (n=15)	0.237±0.054 (n=15)
October	313	0.157±0.008 (n=15)	0.117±0.013 (n=15)
November	348	0.151±0.013 (n=12)	0.118±0.023 (n=126)
December	378	0.088±0.012 (n=16)	0.064±0.009 (n=16)

(Table. 1) generated by gastrocnemius muscle was minimum during the cold of December, maximum during summer (March and April). However, these tensions decreased again at the arrival of next winter. Such influence of season on active tension is due to the fact that during winter, Uromastix undergoes hibernation, that is associated with, decreased metabolic activity, decreased availability of energy, decrease ionic contents[13] and their fluxes and reduced environmental temperature. All of these factors must have been responsible for the decrease in active tension. On the other hand[15] have reported that during winter hibernation, an acidosis occurs in the brain. It has been also reported by[13] that probable increase in acidity might be due to a decrease in bicarbonate contents of muscle. A fall in pH from 7.5 to 6.5 has been reported to raise the calcium binding[16] therefore, it is possible that a similar fall in pH in the uromastix muscle may had reduced the availability of free ionic calcium during contraction. It was therefore, the reason that we observed lesser tension in our experiments during winter. Moreover, there are two main factors that enhanced the active twitch tension in summer, i.e., environmental temperature and the availability of sufficient food for the animal enhancing the availability of energy of activation[7]. Moreover, during summer, increased enzymatic activities, increased availability of creatine phosphate[17], an enhancement in the release of free ionic calcium[18] and the increased availability of other ions[13], have been reported for physiological processes. For these reasons, the height of the active tension curves was found to be minimum during the winter and maximum in summer (Fig. 2).

Table-1: Effect of Season on the Tension Equilibrium Lengths (TEL)

Gastrocnemius Muscles

SEASON		TEL* % Resting Length MEAN ±SE
MONTHS	DAYS	
December	13	100.60 ± 0.6667
January	43	101.29 ± 1.418
February	82	101.69 ± 0.518
March	105	100.82 ± 0.697
April	143	101.04 ± 3.708
June	195	101.36 ± 0.612
October	313	101.08 ± 0.568
November	348	101.53 ± 0.693
December	378	100.60 ± 0.667

***Lengths at which active and passive tensions are equal**

On the other hand, the passive tension curves obtained in winter (Fig. 2) demonstrated a comparatively slow rise below the resting length and lesser values of maximum passive tensions at the highest muscle length. Passive tension generation (Table.1) in the muscle has been shown[19, 20] to be associated with the presence of SEC (at lower length) and with the sarcolemma (at longer lengths). In the light of present study it is suggested that, in addition to the contractile proteins, the non-contractile proteins are also affected by season in term of their quality or quantity. Increased tension generation has been associated with the formation of new contractile proteins during the arousal period and probably also during the spring month of seasonal cycle. Series elasticity has also been proposed to reside in the thin and thick filaments and also in the cross bridges[21]. Therefore, it is assumed that the seasonal changes occurring in the contractile protein contents of gastrocnemius muscle[13] themselves contributes in the changes observed in passive tension curves (Fig. 2). This assumption further strengthened in view of studies done by[22] that an increase in the resting tension of frog skeletal muscle was not due to the connective tissue as commonly thought, but was due to the elastic resistance offered by the myofibrils themselves.

The term "Tension Equilibrium Length" (TEL) has been used by[13] to represent the muscle length at which both the active and passive tensions are equal. It is believed that a change in this parameter would also reflect a change in the contractile and elastic behavior of the experimental muscle. Our results have demonstrated non-significant (P>0.05) changes in the TEL (Table.2) of gastrocnemius muscle through out the whole of the seasonal cycle. This non-significant rise in this parameter towards summer and a fall towards winter indicate that seasonal changes do affect the contractile and elastic elements of this muscle, but not to an extent that can cause significant change in this parameter.

It is concluded that the seasonal variations are responsible for some quantitative and qualitative changes in the characteristics of the contractile (dominant in summer) and elastic elements (dominant in winter) in the skeletal muscles of this hibernating reptile Uromastix hardwickii as reflected by the measured L-T parameters in this study.

REFERENCES

1. Planter WS, Sheilds JL, Purdy FA. Tissue glycogen fractions of the hypothermic rat, hamster and turtle. Amer J Physiol 1964;207(1):42-6.
2. Hyvarinen H. Seasonal changes in the activity of the thyroid gland and the wintering problem of the common shrew (sorex araneus L.). Aquilo Ser Zool 1969;8:30-35.
3. Wenberg GM, Holland J. The circannual variations in the total serum lipids and cholesterol with respect to body weight in the wood chuck (Marmota Monax). Comp Biochem Physiol 1973;44(2):577-83.
4. Said KM, Hussein HK. Seasonal Fluctuation In The Fat Storage Of The Lizard Scincus Officinalis And Its Adaptive Significance To Gonadal Activity. J Egypt Ger Soc Zool 1992;07A:1-15.
5. Johnston IA, Temple GK. Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. The Journal of Experimental Biology 2002;205: 2305-22.
6. Hannah VC, Matthew TA, Sandra LM. Mammalian Hibernation: Cellular and Molecular Responses to Depressed Metabolism and Low Temperature Physiol. Rev 2003;83:1153-81.
7. Penny RK, Goldspink G. Adaptation of the contractile proteins of the desert lizard uromastix micro-lepis. J Uni Kuwait (Science) 1979;6:159-68.
8. Taib NT, Bashir MJ. Histochemical studies studies on the lingual salivary glands of the spiny-tailed lizard uromastix microlepis. Bull Inst Zal Acad Sin (Taipei) 1985;24(2): 203-12.
9. Arsalan M, Samina J, Qazi MH. Seasonal variations in testis of the spiny tailed lizard, Uromastix hardwickii Gray. Biologia 1972;18(1):18-28.
10. Melkumyan LS. The reproductio n period and seasonal changes of the body weight of lizards in the Araks River Basin Ekologiya 1972;3(3):97-8.
11. Zain AM, Zain BK, Rehman AM. Blood electrolytes of a lizard. Pak J Biochem 1969;2 (2): 47-9.
12. Gordon AM, Huxley AF, Julian FJ. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. J Physiol 1966;184:170-92.
13. Azeem MA. Seasonal & temperature variation in the mechanical contraction, strength duration & length tension properties of Skeletal muscle of Uromastix (Ph. D. Thesis) Department of Physiology, University of Karachi. Pakistan. 1992
14. Khalil F, Masseih GA. Tissure constituents of reptiles in relation to their mode of life: Lipid contents. Compar Biochem Physiol 1962;6: 171-4.
15. Malan A, Rodeau JL, Daull F. Intracellular hibernation and respiratory acidosis in European hamster. Journal of Comparative Physiology Biochem Sys Env Physiol 1985;156(2): 251-258.
16. Nakamura Y, Schwartz A. The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. J Gen Physiol 1972;59:22-23.
17. Zimny ML, Gregory R. High-energy phosphates during hibernation and arousal in the ground squirrel. Amer J Physiol 1958;195:233-6.
18. Mukaherjea M. Cation content in the phasic and tonic muscles of frog and its seasonal variation. Ind J Exp Biol 1969;7(4):231-3.
19. Brady AJ. Active state in cardiac muscle. Physiological Review 1968;48:570-600.
20. Hill AV. First and last experiment in muscle mechanics. Cambridge University Press. 1970
21. Suga H. Variable series elasticity accounts for Fenn effect of skeletal and cardiac muscles. Amer J Physiol 1990;258 (part2) R457-R461.
22. Magid A, Law DJ. Myofibrils bear most of the resting tension in skeletal muscle. Science (Wash DC) 1985;230 (4731): 1280-2.

Address For Correspondence:
Dr. M. Abdul Azeem, Department of Physiology, University of Karachi. Karachi. 75270. Pakistan.
Email: azenmu@super.net.pk, azenmu@gmail.com