

MECHANICAL CHARACTERISTICS OF RIB CAGE ELEVATOR MUSCLE OF REPTILE *UROMASTIX HARDWICKII*

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Background: Skeletal muscles are diverse in their contractile properties, with significant differences existing among and within various animal species. Uromastix is one of the desert adapted reptile that possesses unique mechanical characteristics for its skeletal muscles. Additionally, in the absence of diaphragm, this reptile involves one of its chest muscle named recently¹ as Rib Cage Elevator (RCE) muscle that lift the rib cage during active respiration.

Objective: The purpose of this study was to test that increased frequency of stimulation alters the mechanical activity in RCE muscles as well or not and is there any difference in various strips of the RCE muscle of this animal in terms of endurance to high frequency of stimulation, fatigue and which of this strip is mainly responsible for lifting of rib cage during active respiration in this reptile in the absence of diaphragm. **Method:** For this purpose isolated strips of rib cage elevator muscle of Uromastix, (superior, middle and inferior) were used for isometric recordings to compare their mechanical activity. The isometric twitch (Strength 50V, duration 0.5ms and frequency 1Hz) and tetanus (Strength 50V, duration 0.5ms and frequencies, 16, 24, 40, 80, 160 and 240Hz) were recorded with 2 min rest in between each record. The twitch contraction (CT) and twitch and tetanus half relaxation time (1/2RT), were also calculated together with the maximum rate of rise in twitch & tetanic tensions at above frequencies. **Results:** The average values of twitch and tetanic tensions as well as twitch CT, tetanus 1/2RT, rate of rise in twitch and tetanus at varying frequency along with endurance, were greater in inferior and middle strips than that of the superior one. **Conclusion:** The mechanical activity of RCE muscle of Uromastix also depends on the stimulation frequency and mostly significant difference exists in the superior, middle and inferior ones. Therefore, on the basis of greater endurance observed after high frequency of stimulation in the inferior strip of this muscle, it is concluded that it is mainly playing active role in RCE muscle to lift the rib cage during active respiration in Uromastix in the absence of diaphragm.

Keywords: Skeletal muscle, Mechanical properties, Uromastix, Ribcage elevator muscle.

INTRODUCTION

It has been reported that any previous activity done by the skeletal muscle may affect its performance². The stimulation frequency force relationship is one of the characteristics of skeletal muscle that is also changed if obtained after some activity. It has been observed that muscle force increases with increase in stimulation frequency up to an optimal and later decreases at higher frequencies in mammalian skeletal muscles. However, the amount of tension produced by an individual muscle fiber depends solely on the number of interacting cross-bridges. Hence, the amount of tension or force produced by the skeletal muscle as a whole certainly depends upon the frequency of stimulation and the number of muscle fibers stimulated.

Earlier studies showed that repeated activation of muscle induces both processes resulting in either decreased performance (fatigue) or enhanced performance³. Fatigue is described as the failure to maintain the required or expected force or power output⁴. While, Potentiation is the increase of force at sub-maximal levels of activation due to repeated low-

frequency stimulation (staircase potentiation) or of a previous tetanic contraction (post-tetanic potentiation) as reported by Krarup⁵. Thus, both fatigue and/or potentiation are responsible to change the mechanical characteristics of skeletal muscle. Potentiation of force by previous activation is caused by increased rates of phosphorylation of the myosin light chains (MLCs) as investigated by Manning & Moore^{6,7} leading to an increased sensitivity to Ca²⁺. Further, increase of frequency will lead to decreased availability of Ca²⁺ due to start of fatiguing process in muscle. So increased frequency will lead to rapid binding of actin and myosin with increase force generation. On the other hand stimulation above critical frequency can start fatigue in the muscle, resulting in slowness in actin and myosin interaction thus decreasing the force.

The Uromastix hardwickii exhibits maximum locomotory speed as a reptile at high desert temperature. This characteristic has been attributed to specific contractile characteristics of its skeletal muscles⁸. However, these characteristic may differ in a single animal for different muscles as well. Force generation in muscle is generally considered to

be the function of myosin cross-bridges that are attached to the actin filaments⁹. Therefore, difference among muscles in the recorded force is actually due to a difference in the interaction speed of cross bridges with actin in them. On the basis of above literature, the present study was designed to investigate the changes in the mechanical characteristics of isolated RCE, a recently identified¹ muscle on high frequency of stimulation and fatigue in terms of twitch, tetanic tension, rate of rise in tension and CT, 1/2RT. The comparison of these characteristics among different strips is aimed to find out the main strip of RCE involved in lifting the rib cage during active respiration in this animal in the absence of diaphragm.

MATERIALS AND METHODS

Six *Uromastix hardwickii* of both sexes were used. Fresh animals were decapitated according to the

standard ethical standards and used for the isolation of RCE¹. The RCE muscle has been divided into three parts according to its involvement in locomotion as well as rib cage elevation. The superior and middle parts have been found to be involved in the fore limb movement for locomotion¹ and the inferior part, in the rib cage elevation (Fig. 1).

The inferior part was dissected out by removing it from the condyle of humerus (point of origin) and the last rib (point of insertion). The muscle was further separated into superior, middle and inferior strips according to their anatomical positions seen visually. These strips were then immediately transferred to a fresh reptilian buffer solution having the following composition.

Reptilian buffer solution, NaCl 100mM, KCl 2mM, CaCl₂ 1.8 mM, Na₂HPO₄ 5.8mM, KH₂PO₄ 1.2mM.

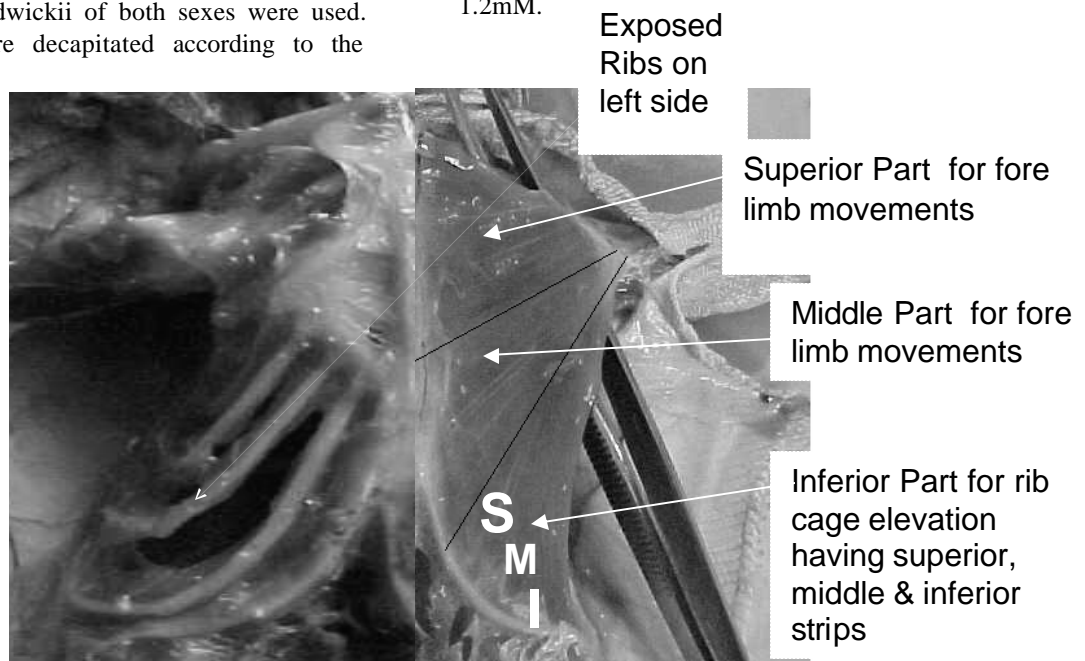


Fig. 1: Ribcage elevator muscle: S=Superior strip, M=middle and I=Inferior strip *Uromastix hardwickii*

MECHANICAL RECORDINGS:

RECORDING EQUIPMENTS:

Silver chloride electrodes, Muscle bath, Isometric transducer (Cat. No. 50-7913), Thermostatic bath (Model RMT6, Cat. No. 232-030), Oscillograph (Harvard apparatus Ltd. (U.K) Cat. No. 50-8622), Macro-manipulator (C.F. Palmer, London), Stimulator (Palmer Bioscience. CAT. No: 220).

RECORDING PROCEDURE

For this purpose thermostatic bath was filled with distilled water and connected to muscle bath with the help of two tubes that helped to circulate water in both the internal and external chamber of muscle

bath. The internal chamber was filled with reptilian buffer solution. The muscle strip was fixed in the internal chamber by its proximal end with the pin provided. The distal end of the muscle having tendon was attached by means of hook and thread which was passing through a pulley to the force transducer. This transducer was installed on a macro-manipulator for its vertical adjustment. This force transducer connected to the oscillograph via lead and the pair of stimulating electrode was placed beneath the muscle for stimulation through DC pulse stimulator.

Zero line of the channel amplifier was also adjusted. For this purpose the paper speed was set at 1mm/sec and amplitude of the channel amplifier was set at zero. Then by moving the position knob a base

line was fixed. Later, the gain of the channel amplifier was adjusted to maximum. After the adjustment of gain if there is any change in the base line, it was adjusted through D.C knob of interface to bring pen to its original base line. Later, Calibration was obtained by hanging 5gm of weight with the leaf of transducer at different sensitivities of channel amplifier to record pen deflections at specific chart speed. These were used later for the calculation of tension and time parameters.

For the determination of resting length, muscle length was increased gradually from flaccid towards stretched length with the help of macro-manipulator till maximum pen deflection was recorded on stimulation.

After adjusting muscle length (resting) the isometric twitch and tetanii were recorded from the experimental muscles by providing twitch and tetanic stimulations (50V strength, 1Hz frequency, 0.5ms duration). Tetanic records were obtained every 2 minutes at stimulation frequencies of 16, 24, 40, 80, 160 and 240 HZ. After 10 minutes of recovery, same strip was fatigued by using frequencies of 1 HZ, 50V strength & 0.5ms duration.

The individual data was used for the calculation of mean \pm SE. One-way ANOVA was performed on parametric data, to compare differences among strips. The level of significance was 0.05.

RESULTS

The results showed that the isometric twitch and tetanic tension was greater in the inferior strip of the RCE muscle than its superior and middle ones. These tensions were found to increase gradually on increasing the frequency of current for muscle stimulation. However, their maximum value was recorded at 80Hz except in the superior strip where these tensions were maximum at 24Hz (Table 1 & 2). However, all the twitch and tetanic tensions recorded from these strips have demonstrated non significant difference among each other. The results have also been presented to show a comparison between time dependent parameters which included twitch CT, twitch and tetanus 1/2RT (Table 3 & 4).

Table-1: Comparison of the average values of twitch tensions (Kg/cm²) in between inferior, middle and superior strips of Rib Cage Elevator muscle of Uromastix hardwickii.

TWITCH TENSIONS		
Inferior	Middle	Superior
2.737 \pm 0.778 (6)	2.008 \pm 0.714 (6)	1.654 \pm 0.555 (6)

P > 0.05

Table-2: Comparison of the average values of tetanic tensions (kg/cm²) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

FREQUENCY	TETANIC TENSIONS			P
	INFERIOR	MIDDLE	SUPERIOR	
16 Hz	3.993 \pm 1.233(6)	2.58 \pm 0.758(6)	1.986 \pm 0.749(6)	P>0.05
24 Hz	5.564 \pm 1.608(6)	4.868 \pm 1.412(6)	2.525 \pm 1.000(6)	P>0.05
40 Hz	7.101 \pm 1.932(6)	6.263 \pm 1.602(6)	2.515 \pm 1.108(6)	P>0.05
80 Hz	7.711 \pm 2.167(6)	6.636 \pm 1.464(6)	2.448 \pm 1.097(6)	P>0.05
160 Hz	6.167 \pm 1.865(6)	5.924 \pm 1.460(6)	1.999 \pm 0.865(6)	P>0.05
240 Hz	6.005 \pm 1.895(6)	5.713 \pm 1.339(6)	2.443 \pm 1.099(6)	P>0.05

Table-3: Comparison of the average values of twitch contraction and half relaxation times (Sec) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

PAREMETERS	MUSCLE STRIPS			
	INFERIOR	MIDDLE	SUPERIOR	P
TWITCH CONTRACTION TIME	0.048 \pm 0.001(6)	0.048 \pm 0.008(6)	0.045 \pm 0.005(6)	P>0.05
TWITCH HALF RELAXATION TIME	0.042 \pm 0.005(6)	0.043 \pm 0.004(6)	0.032 \pm 0.001(6)	P>0.05

Table-4: Comparison of the average values of tetanus half relaxation times (Sec) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

	MUSCLE STRIPS			
FREQUENCY	INFERIOR	MIDDLE	SUPERIOR	P
16 Hz	0.050± 0.007(6)	0.050± 0.003(6)	0.035 ±0.005(6)	P>0.05
24 Hz	0.053± 0.004(6)	0.058± 0.004(6)	0.041± 0.007(6)	P>0.05
40 Hz	0.055± 0.004(6)	0.063± 0.004(6)	0.044 ±0.005(6)	P<0.05
80 Hz	0.066± 0.005(6)	0.072 ±0.004(6)	0.047 ±0.007(6)	P<0.05
160 Hz	0.070± 0.01(6)	0.071± 0.01(6)	0.044± 0.01(6)	P<0.01
240 Hz	0.068 ±0.005(6)	0.067± 0.004(6)	0.046± 0.007(6)	P<0.05

Table-5: Comparison of the average values of rate of rise in twitch (Kg/cm2/sec) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

MUSCLE STRIPS			
INFERIOR	MIDDLE	SUPERIOR	P
66.8432 ±17.49(6)	67.081± 24.847(6)	46.297± 13.008(6)	P>0.05

Table-6: Comparison of the average values of rate or rise in tetanus (Kg/cm2/sec) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

	MUSCLE STRIPS			
FREQUENCY	INFERIOR	MIDDLE	SUPERIOR	P
16 Hz	56.000 ±11.599(6)	65.042±28.012(6)	43.918±16.010(6)	P>0.05
24 Hz	52.714± 11.467(6)	55.643±20.360(6)	43.122±15.386(6)	P>0.05
40 Hz	67.156± 18.110(6)	65.876±23.223(6)	36.061±14.453(6)	P>0.05
80 Hz	72.136± 19.859(6)	72.615±22.800(6)	41.648±16.024(6)	P>0.05
160 Hz	64.877 ±19.778(6)	74.616±24.252(6)	41.754±15.196(6)	P>0.05
240 Hz	60.941±22.935(6)	78.283±27.079(6)	66.531±15.224(6)	P>0.05

The twitch CT was greater in inferior and middle strip. Where as twitch 1/2RT was maximum in middle strip. These values have represented statistically non significant differences among them. The tetanus 1/2RT has been found to increase initially on increasing the frequency of stimulation which later decreased at higher frequencies being maximum at 160Hz and decreased at 240Hz. In middle and superior strips the maximum values were found at 80Hz. However, statistically there was significant differences among these time parameters in all the strips at the stimulation frequencies of 16 to

24 Hz, while it was non-significant at 40, 80, 160 & 240Hz (Table 4).

Time dependent tension parameter, i.e., the maximum rate of rise in twitch and tetanic tensions have been presented in Table 5 & 6 respectively.

Maximum rate of rise in twitch was observed in the middle strip. While the maximum rate of rise in tetanus was gradually increased with increasing stimulation frequency. In the inferior strip, the maximum value was found at 80Hz. In middle and superior strip maximum value was observed at 240Hz. However, no significant difference was

observed among these strips at these frequencies of stimulations.

The time required to fatigue was also determined for all the strips (Table 7).

Table-7: Comparison of the average values of time to fatigue (Minutes) in between superior, middle and inferior strips of Rib Cage Elevator muscles of Uromastix hardwickii.

Muscle strips		
Inferior	Middle	Superior
6.800 ± 0.800 (6)	6.100± 0.331 (6)	2.000 ± 1.379 (6)

P>0.05

Result demonstrated that inferior strip has the maximum resistance to fatigue than the middle and superior ones because of greater average time taken for complete fatigue, after high frequency stimulation. However, Statistically, the average values of time to fatigue determined for three strips were non-significantly different from each other.

DISCUSSION

The present study was aimed to investigate the mechanical characteristics of superior, middle and inferior strips of Uromastix RCE muscles, on different frequencies of stimulation and their endurance to fatigue. The method used in this study has also been validated in numerous other studies on human and animals other than Uromastix.¹⁰⁻¹³

On the basis of comparison of the results obtained on the superior, inferior and middle strips of RCE muscle of Uromastix, the inferior strip has demonstrated greater contractile activity both in terms of twitch and tetanus. In our opinion, anatomically, since this inferior strip is thicker than the middle and superior ones (Fig-1), therefore, it must be possessing greater number of muscle fibers. This difference has produced greater force in the inferior strip (Table-1). In addition, a comparison of these strips with respect to increasing frequency of stimulation has demonstrated that the tetanic tensions in both the inferior and middle strips increased gradually from a minimum to their peak between 16 to 80Hz of stimulation frequencies which later declined at 160 and 240 Hz. However, the superior strip of RCE muscle produced maximum tetanic tension at lower frequency of 24Hz which later varied between 40 to 240Hz (Table 2). These results indicate that on increasing the frequency of stimulation, the recruitment of muscle fibers was better in inferior and middle strips probably due to greater number of muscle fibers and thickness of

these strips, while in case of superior strip maximum recruitment occurred at 24Hz. It indicates that maximum number of available muscle fibers were involved in tetanization process at this frequency in superior strip having probably less number of muscle fibers in it, being the thinner strip.

Further, the difference in force generation among the superior, inferior and middle strips of RCE muscle may also be related to a difference in their whole muscle length. Recently³ has demonstrated greater post-tetanic potentiation on increasing the muscle length in rat medial gastrocnemius muscle. Although, the present study was not done on increasing the length of individual strips, but even then, it is expected that greater the length of strip of a muscle, greater should be the number of sarcomeres per muscle fiber. Rassier et al¹⁴ demonstrated that if fibers within a given muscle have different average sarcomere lengths at a given muscle length, peak force would be reduced and the plateau would be broader, compared with fibers of identical sarcomere lengths within a given muscle. In this connection, it is important to note that the superior strip of RCE muscle used in this study was having different length of its muscle fiber as this strip (Fig-1) originates exactly at the condyle of humerus and fans to insert in the lower most rib. In contrast the length of the middle and inferior strips though short but probably having thicker fibers of more or less same length as they originate about mid portion of the RCE's superior strip through the myo-tendon junction at their origin. However, a detailed histological/morphometric study is necessary to reveal the exact details to further ascertain their role in ribs elevation along with the findings of present study in the RCE muscle of reptile Uromastix.

The time parameters, i.e., twitch CT and 1/2RT were not different, statistically, among all the strips of RCE muscle of Uromastix (Table-3). While, there was statistically significant difference among them for tetanus half relaxation times recorded at frequencies of stimulation between 40 and 240 Hz (Table-4). This difference clearly indicate that the RCE muscle is also principally similar to the skeletal muscles of other animals and human in terms of tension and time related mechanical response. However, according to the Table 4, the tetanus 1/2RT was maximum at 160Hz in the inferior strip and at 80 Hz in the middle and superior strip of RCE. It means our suggestion regarding the availability of greater number of muscle fibers in inferior strip may be graded among all the three as: inferior<middle<superior in terms of their contractile response observed. This suggestion also get strength from the obtained maximum average values of tetanus 1/2RT obtained at 160HZ in inferior and at

80Hz in middle and superior strips of RCE muscle. It is important to note that among these maximum values of tetanus 1/2RT, the average value of superior strip is the least one.¹⁵ Working on skinned frog skeletal muscles fibers suggested that the slow phase of relaxation is influenced by the degree of sarcomere homogeneity and rate of Ca²⁺ dissociation from thin filaments. The fast phase of relaxation is in part determined by the level of Ca²⁺ activation. In the present study the greater tetanus 1/2RT observed in inferior and middle strips at optimal frequency in comparison with the superior strip of RCE muscle probably indicate the availability of homogenous sarcomeres along with slow dissociation of Ca⁺⁺ from the actin myosin complex during relaxation in inferior and middle strips of RCE muscle.

Vøllestad et al¹⁶ reported that relaxation rate is an important factor in determining the contractile response of the muscle fibers during isometric contraction and this slowing of relaxation may reflect a transient larger rise in P_i and H⁺ levels due to a disturbance of the aerobic recovery after applying the high-frequency pulse train. At the cellular level, the relaxation process is regulated by enzymes controlling the rate of Ca²⁺ reuptake in the SR and, possibly, also the rate of cross-bridge detachment¹⁷. Hence, in the present study, the greater average values of tetanus 1/2RT in middle strip than the inferior and superior one (Table 6) probably reflects a difference in middle strip than superior and inferior one regarding changes at cellular level in terms of P_i and H⁺ levels on tetanization related aerobic recovery¹⁶.

In addition, in the present study, the average values of rate of rise in twitch and tetanic contractions demonstrated lesser values in superior strips of RCE muscle than the inferior and middle ones, representing a probable low rate of cross bridge interaction towards the peak of tension in superior strip at all the frequencies except at the highest 240HZ. However, these values were still lower than those obtained from middle strip at same frequency.

The average values of endurance, i.e. time to fatigue after high frequency of stimulation provided to three strips of RCE muscle (Table 7), demonstrates endurance grading as: Inferior>middle>superior. This grading indicate a probable availability of comparatively slow twitch fiber population in inferior and middle strips than that of superior one. However, this assumption should be tested on histochemical basis for confirmation regarding these strips of RCE muscle.

According to Bigland-Ritchie et al¹⁸ the motor unit firing rate during repetitive isometric contraction remains low. In addition¹⁹ a study on human skeletal muscles reported that at the lowest

stimulation frequencies the energy cost is high, i.e., six times than required during maximal stimulation. This reasoning have also been adopted in other studies to observe high energy cost for isometric twitch and short intermittent tetanii than for tetanic contractions of longer durations^{4,20}. It means that in the present study, the less twitch and tetanic tensions as well as less rate of rise of tensions recorded for the superior strip may be due to its high energy cost for tension generation than those of the inferior and middle strips of RCE muscle of Uromastix.

On the basis of above discussion it is clear that in RCE muscle of Uromastix which is identified recently¹ has three strips with different mechanical characteristics and on the basis of differences in mechanical characteristics reported in this study for the three strips of REC muscle, it is confirmed that the inferior strip exerts better role in rib cage elevation during active inspiration in the absence of diaphragm in this reptile Uromastix.

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