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Original Research

Seasonal effects on water and osmotically-active ionic contents (Na^+, K^+ & Cl^-) of skeletal muscles of the spiny-tailed lizard, *Uromastix hardwickii.*

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Abstract

To assess the seasonal effects on water & osmotically-active ionic contents (Na+, K+ & Cl-) of skeletal muscles of the spiny-tailed lizard Uromastix hardwickii.

Fresh animals (adults) of both the sexes were used in all the experiments, and the gastrocnemius (skeletal) muscles were dissected out, reptilian buffer solution was used to keep dissected muscles moist & used during experimentation. The muscle sample solutions were prepared by heating/drying and incinerating/ashing the muscle tissues, treating with acid and deionized water respectively. These samples were then used for the estimation of Na+, K+ & Cl- ions. The water content was found as percent wet muscle weight.

The average mean values of water content and these osmotically active ionic contents were found to fall significantly ($P<0.05$) from peak winter towards peak summer & vice versa.

The seasonal effects of these parameters, studied for the first time in the skeletal muscle of Uromastix hardwickii, displayed changes as the animal passed from one seasonal phase to another. Hence seasonal changes are invariably associated with changes in environmental temperature, and definitely responsible to change the active behavior and homeostasis of these animals from summer towards winter by variations in blood borne humoral and hormonal substances along with significant changes in osmolality and tissue water contents.

INTRODUCTION

The reptile *Uromastix* is a typical desert-adapted animal with a spiny tail that resembles a wall lizard (1). They live in burrows, are generally herbivorous (2), and are capable of absorbing water through their skin (3). They show their normal activity at temperatures of about 32 – 36 ^{O}C in summer. At lower temperatures (e.g. $16^{O}C$ & below) during the cold winter, however, they become markedly sluggish, calm, and quiet; and undergo sleep in their burrows, known as the hibernation period of animals (1,3)

Osmolality is the concentration of osmotically active particles in a solution (4). Almost half of the osmotically active ECF particles are Na^+ ions (including K^+ & Cl^-) which regulate ECF volume (5). Very little information is available regarding the ionic contents in *Uromastix,* except for some information about plasma ionic contents during active & hibernating periods of the animal (6), and differences in the Na^+ and K^+ ion contents in the skeletal, and cardiac muscles of *Uromastix hardwickii* (7).

Seasonal changes in some tissues and activities of *Uromastix hardwickii* have also been reported in literature, like variations in testis size (8), changes in body weight (9), changes in plasma electrolyte concentration (10), and variations in the mechanical contraction, strength, duration, and length tension properties of skeletal muscles (2,7) have been reported. But seasonal effects causing changes in the ionic contents (osmolality) of skeletal muscles of this species of the animal have not yet been reported. Hence the purpose of this study was to assess the seasonal effects on water & osmotically-active ionic contents (Na^+, K^+ & Cl^-) of skeletal muscles of this spiny-tailed lizard *Uromastix hardwickii*. The changes in osmolality parameters, esp. Na^+ followed by Cl^- & water content have a greater chance to maintain electrolyte and water balance not only in animals but in humans as well (4,11,12).

MATERIALS AND METHODS

The study was carried out in peak winter months of December & January and peak summer months of June & July; at University of Karachi, Pakistan.

Fresh animals (adults) of both the sexes were used in all the experiments. In the laboratory, the animals were kept at room temperature (25^OC). Since their biochemistry is reported to change with season (10), all the animals of a fresh batch were used up within a week (2,13,14).

For experimental purpose,s the animals were killed by decapitation and the gastrocnemius (skeletal) muscles of both the limbs were dissected out (2,7,14).

For the measurement of ions (Na^+, K^+ & Cl^-), freshly dissected muscles were shifted in a watch glass, after being weighed , to keep in the oven at 80^OC over night; to dry them before the start of the incineration/ashing procedure. The reptilian buffer solution (extracellular solution) used in the experiments was of the composition (in mM): 124 NaCl, 3.8 KCl, 1.8 $CaCl_2$, 5.8 Na_2HPO_4, 1.2 KH_2PO_4, with pH = 7. 2 (15, 16, 17).

For ashing purposes, the dried muscles were shifted to porcelain crucibles and were incinerated at 550 ^OC for 8 hours in a Chinese muffle furnace, model RJM from HE GE ZHENG Company. The ashes obtained were then treated with 1 ml HCl/gm wet weight of the muscle in each crucible to dissolve all the organic material and the excess acid was removed by heating the crucible on a low flame. These samples were then kept again in the furnace at 550 ^OC for 2 hours. After the second ashing, the ash was retreated with 25% HNO_3 (1ml / gm wet weight). The excess of acid was again removed by heating the sample on a low flame. The samples were then re-kept in the furnace at 550 ^OC for another 2 hours. After this third ashing, the samples were dissolved in 5 ml of deionized distilled water and were heated on a low flame to dissolve all the ions present in the sample. These samples were then used for the estimation of Na^+, K^+ & Cl^- ions (7).

Flame emission spectrophotometer, Corning Model 410 was used, which measured the concentration of Na^+ & K^+ in mMol / L (18) (1 mM of Na^+ = 1 mEq of Na^+/ valence: 1 = 1 mEq of Na^+, that in turn equals to 1 mOsmol of Na^+; in the same manner 1 mM K^+ = 1 mOsm and also 1 mM of Cl^- equals to 1 mOsm) (11,19). The technique used to measure Cl^- in mMol/L, is Photometric Colorimetric Test TPTZ method (20,21).

Water content of the gastrocnemius muscle was calculated as percent wet muscle weight (wet muscle weight – dry muscle weight / wet muscle weight, then taking out its percentage) (22).

All of the calculations including multiplications, divisions, averages, standard errors and 't' tests and P-values done in the present work were carried out on MS Office Excel and Minitab version 13.30. The one way analysis of variance (ANOVA) was evaluated. P-Value approach was adopted which suggested the evidence in favor of, or against, the null hypothesis; keeping in consideration the degree of freedom for variation associated "between the treatments" (peak winter & summer) and "within the treatment". Hypothesis was rejected for P-Value smaller than 0.05 (23).

RESULTS

Water content (% wet muscle weight)

The average values of water content were found to fall significantly($P<0.025$) from peak winter (the months of December & January) towards peak summer (June & July), as shown in Table 1. The fall of water content however, was only 5% (Table1).

Na^+ (mOsm/L)

The average values of Na^+ (mOsm/L) mentioned in Table 1 were found to decrease significantly($P<0.025$) from peak winter towards peak summer, and this decrease was calculated to be about only 4%.

K^+ (mOsm/L)

The average values of K^+ (mOsm/L) (Table 1), were found to decrease significantly($P<0.025$) in between peak winter & peak summer, and showed a 12% fall in peak summer.

Cl^- (mOsm/L)

The average mean values of Cl^- (mOsm/L) (Table 1), decreased significantly ($P<0.025$) from peak winter towards peak summer, and demonstrated only a 1% change towards peak summer.

Table 1. Seasonal Comparison of Water Content and Osmolality parameters (Na+, K+ and Cl-) of Gastrocnemius muscles of Uromastix hardwickii obtained during *Peak Winter and **Peak Summer months of a Seasonal Cycle

PARAMETERS	Peak Winter Mean ± S.E.M.		Peak Summer Mean ± S.E.M.		Significance Level Winter Vs Summer
Water Content (%Wet muscle weight)	77,88	±1.48	74,10	±0.68	P < 0.025
Osmolality of Na^+ (mOsmol/L)	141,33	±1.87	136,36	±0.69	P < 0.025
Osmolality of K^+ (mOsmol/L)	4,29	±0.21	3,83	±0.07	P < 0.025
Osmolality of Cl^- (mOsmol/L)	103,00	±0.36	102,08	±0.21	P < 0.025

P < 0.025 denotes the significance values
* Peak winter temperature 200C and ** Peak Summer temperature 350C

DISCUSSION

Water content (%wet muscle weight)

The average values of water content in the skeletal muscle tissue of the Uromastix, showed considerable seasonal variations, and denoted a significant fall in between peak winter months December & January, and peak summer months June & July; as shown in Table 1**.** Hence it is evident that water content in the muscle tissues displayed changes as the animals passed from one seasonal phase to the other. Also worked on was the seasonal changing effects on the same parameter but in other tissues (24) like thebrain, kidneys & blood serum; and in other species of the reptilessuch as the Egyptian cobra, and observed related results.

Osmotically active ionic contents

a) Na^+ (mOsm/L): Its average values showed considerable changes with seasonal variation and denoted a significant fall on comparison of average values obtained in peak winter months December & January and with peak summer months June & July (Table 1) (24), These changes were evident in cobra snakes, (25) lizards, Scincus Scincus and (26) in cane toads & bullfrogs, we observed Na^+ as one of the important electrolytes of the tissues of the animals, Its concentration changes with season; hence confirming our findings in the skeletal muscles of Uromastix hardwickii.

b) K^+ Content (mOsm/L): The average values were found to vary with seasonal phases and were significantly higher in peak winter December & January as compared to peak summer June & July (Table 1). Hence, these seasonal variations of K^+ content reflect its loss in summer. (24, 25, 26) Also observed was the related results in other species of animals, which support our observations in Uromastix hardwickii.

c) Cl^- Content (mOsm/L), in the study showed considerable seasonal changes in its average values from peak winter December & January towards peak summer June & July. It showed significant fall from winter towards summer (Table 1). These changes indicated the approach of summer probably shifting Cl^- in between ECF and ICF against the changes in Na^+ and K^+ ions in the body fluids of this animal. [26] We observed significant variations in Cl^- content with temperature changes in all the tissues (except in liver) in air-breathing ectotherms (cane toads & bullfrogs). This confirmed our findings about the Cl^- content in the skeletal muscles of Uromastix.

It is important to mention that seasonal changes are invariably associated with changes in environmental temperature. The results obtained in winter & summer seasons regarding water and osmotically active ionic contents (Na^+, K^+ & Cl^-) of the skeletal muscles of the Uromastix hardwickii, are analogous to changes in experimental temperature effects observed by other workers in other animal species. (26) Studies showed increased water content, Na^+ & K^+ contents in the cells of frogs on cooling from 30 – 20 ^{O}C. (19) There was a significant increas in Na^+ content in the skeletal & cardiac muscle tissue cells by the effect of hypothermia in frogs, and also observed variations in Cl^- content with temperature changes in all the tissues in the same animal, except its liver. Although seasonal variations are not reported for water content & osmolality parameters of skeletal muscle of lizards, one of the studies performed by (23) demonstrated significantly increased Na^+ in the brain and also K^+ rise in brain, blood serum & kidney of Egyptian cobra during hibernation. (12) Analyzed the changes in water content & electrolyte concentrations, esp. rise of plasma Na^+, along with the rise of lactic acid, released from active skeletal muscles in humans.

All of the above mentioned references support our findings of seasonal changes in the water content & osmotically active ions (Na^+, K^+ & Cl^-), studied for the first time in the skeletal muscle of *Uromastix hardwickii*. The same osmolality parameters, along with other parameters studied in humans are also required to maintain electrolyte & water balance in the tissues, including skeletal muscle; which is an excitable tissue like nerve, and these ions are also responsible for maintaining resting membrane potentials in excitable tissues in humans and animals (11,12,16). Our study on the skeletal muscle tissues of this animal has revealed that ionic concentrations in the animal studied are very much relevant with humans, hence hints to expand the further research through the living tissues of this animal;

which can't be performed on living humans beings. This animal is desert-adapted & very much resistant to the conditions of food shortages and lives for several days without any food water because of it's fat storages(17).

CONCLUSION

Seasonal changes in all of the above mentioned parameters of the skeletal muscle of Uromastix hardwickii are definitely responsible for the change in the active behavior & homeostasis of these animals from summer towards winter by reducing the blood borne humoral and hormonal substances along with increased osmolality and body water.

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