



Original Research Article

Cellular lactate and pyruvate are key intermediates for intra-cellular energy metabolism regulated by Lactate dehydrogenase (LDH) in age-related phenomenon

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ABSTRACT

Ageing in animals is accompanied by changes in cellular function. Although the cause of aging appears to be multifactorial, the alterations in cellular functions are the consequence of changes in biochemical composition of cells since a number of enzymes and metabolites regulate the overall activities of the cell. There is a great deal to be known about the interrelationship between growth, sexual maturity and aging in animals. The poikilothermic vertebrates would serve as suitable animal models in which these parameters could be easily manipulated through variation. In the present study we have used the non-mammalian vertebrate common Indian toad to find the glycolytic enzyme activity (Lactate dehydrogenase-LDH) during ageing. We have investigated the LDH activity, pyruvate and lactate content in liver and muscle and compared if there is any biochemical distinctiveness in the ageing pattern.

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1. Introduction

Aging is the process of senescence and is not an uncommon word in scientific literature. Certainly a clockwise process that inherent in the theories of programmed ageing, triggering the necessary gene suitability in the individual cells of an organism at the appropriate stages of its life.¹ All living things are subject to turnover, or physiological regeneration. There are dynamic equilibrium in the resultant of the relative rates of degradation and synthesis at the molecular, organelle and cellular levels of organism.² Unlike rapid senescence (happens in male pacific salmon and marsupial mice, where there is sudden loss of function after reproduction), the gradual senescence occurs in most vertebrates. The cause of aging appears to be multifactorial. Several theories have been proposed to explain the phenomenon of aging, while the programmed theories believe that aging is genetically programmed.³ The free radical theory of aging originally proposed by

Harman,⁴ which states that ageing is due to oxidative stress caused by accumulation and damaging effect of free radicals. The process of determination of age related to physiological and biochemical observations in lower mammals. The metabolic rates in various tissues correlate with the aging pattern.

Even though data on age related changes in the morphometric and biochemical components of different tissues such as liver, muscle, brain, kidney of mammals have been accumulated, there is paucity of literature on non-mammalian vertebrates, especially on enzymatic activity (lactate dehydrogenase), pyruvate and lactate are very limited.⁵ Considering the indeterminate growth pattern exhibited by most poikilothermic vertebrates, it is worthwhile to have comparative study of age related biochemical changes in the skeletal muscle and liver of common Indian toad, *bufo melanostictus*.

To find out, whether there is any biochemical distinctness in the aging pattern, the major glycolytic enzyme (anaerobic) LDH activity, pyruvate and lactate content in

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the muscle and liver of common Indian toad have been used as specific parameters. The liver is considered as the living workshop owing to its involvement in almost all metabolic processes of the body.⁶ The role of liver in the synthesis, maintenance and degradation of metabolites is well known. Being the chief organ of glucose storage in the form of glycogen and releasing glucose during stressed conditions through glycogenolysis, is the major site of gluconeogenesis.⁷ Various groups used liver tissue as suitable model for study of aging.⁸ Liver and muscle contain most of the glycolytic enzymes catalysing various metabolic pathways of carbohydrate metabolism. The skeletal muscle is a dispersed organ system. Moreover, as tissues for anaerobic carbohydrate metabolism, liver and skeletal muscle do crucial independent role.

1.1. Aim and objectives of the study

In the present study, the aim was to investigate the major glycolytic enzyme (anaerobic) LDH activity and the pyruvate-lactate content in skeletal muscle and liver of common Indian toads. This aim was to find out whether there is any biochemical distinctness in the aging pattern.

2. Materials and Methods

2.1. Animals

The present study with common Indian toad *Bufo melanostictus* of both sexes having various body weights were approved by Institutional Animal Ethics Committee. These experimental animals belong to class- Amphibia, sub class- Anura, order- Protocoels, family- Bufonidae. This species is not listed in the “Red Book” of IUCN (International Union of National Resources)

2.2. Animal collection and maintenance

The animal used for this study were easily available locally and usually they remain hidden during day time and come out for searching food in night (nocturnal habit) and mainly they are insectivorous. They don't take dead animal as food. The sex can be distinguished by the presence of single median vocal cord under the throat and swollen glandular thumb-pad on forelegs (during breeding season) of male. Generally females are larger size than males. The animal of both sexes and of various sizes (body wt. 9 gram to 114 gram) and S-V length of range 3.2 to 11.5 cms were collected from the Berhampur University campus. Collection was done during evening time and randomly different sizes and of numbers from 7 to 10.

After collection, toads were maintained inside the wire-netted glass cages where sand bed was prepared sprinkled water. They were acclimatized for a period of at least two days and used for experiment in third day.

2.3. Estimation of morphometric parameters

Based on body weight, male toads were conveniently divided into two groups such as younger (body weight ranging from 10-13g) older are 30g and above. Females were divided into three groups such as more 60g regarded as older, 30-60 g is middle and less than 30g were younger. The estimation of all the morphometric and biochemical parameters were completed with all the batches of animal of various size and of different sex also. The weight of the animal was determined in a chemical balance and the Snout-Vent (S-V) length using measuring tape.

2.4. Tissue processing and estimation of lactate and pyruvate

The animals were pithed by piercing a pointed needle just posterior to occipital region. Immediately, the thigh muscle and the entire liver were dissected out in ice-cold Amphibian Ringer (KCl 140 mg, NaCl 6.5g, CaCl₂ 120mg, NaHCO₃ 100 mg/ Litre), pH 7.4). The adherent connective tissues, blood vessels and nerves were removed and each tissue was blotted off with whatmann filter paper no.1. Then the tissues were taken for various biochemical parameters after making homogenate.

For LDH activity, 2% homogenate was prepared in ice cold water using Remihomogenizer. Then LDH activity was measured following method of Cabaud et al., (1958). Briefly, the assay mixture consisted of 0.5ml phosphate buffer (pH 7.4), 0.5 ml pyruvate substrate (sodium pyruvate, BDH). A blank without substrate was run simultaneously along with the samples. All the tubes were incubated for 45 minutes at 37 deg which gives optimum activity. After incubation, the residual pyruvate is complexed in alkaline medium with 2-4-dinitrophenylhydrazine (BDH, LR) to form the hydrazine which absorbs maximally at 530 nm. The enzyme activity was expressed as units of LDH as described by Oser.⁹

Extraction and estimation of Lactate and Pyruvate: 100mg of muscle and liver were taken separately and homogenized using Remihomogenizer with 2.5 ml 10% cold TCA (BDH, Analar) and centrifuged in Remi centrifuge at 4 deg. The supernatant was collected in a tube. To the residue 2.5 ml cold TCA was added and stirred again. It was centrifuged for a minute and the supernatant was added to the previous collection tube. The volume of the supernatant was measured for each tissue and kept in 4 deg for biochemical estimation.

Estimation of lactate content was done following the method of Barker and Summerson¹⁰ using Lithium lactate (BDH) as standard. Optical density was taken at 560 nm in spectrophotometer. The lactate content was expressed in ug/g tissue.

Estimation of pyruvate content of the sample was determined Friedmann and Haugen¹¹ using Lithium

pyruvate (BDH) as standard. OD was measured at 520 nm and pyruvate content was expressed in $\mu\text{g/g}$ tissue.

Lactate/pyruvate ratio was calculated dividing the values of lactate by that of pyruvate of each tissue.

Statistical analysis: The statistical analysis of all the samples was done using students t-test and the confidence level (P) was found out using t-table (Bishop, 1966).¹² P value at 0.05 levels and below was considered as significant and above was not significant.

3. Results

In the present study, an attempt has been given to find out the age and sex related changes in the hepatic LDH activity and skeletal muscle LDH activity along with Lactate/Pyruvate ratio in both the tissues.

3.1. Morphometric parameters

As pointed out in the material method section, the body weight and snout to vent length increases with advance age in both the sexes of toad (Table 1). Also the total weight of liver increased significantly ($p < 0.001$) in both the sexes with the increase in age (Table 2).

3.2. Age related alterations in liver

The hepatic lactate dehydrogenase activity in male toad increased significantly ($p < 0.001$) in group-II as compared to younger group I, whereas in female there was no significant increase in group II and group III. However, the comparison between male and female indicated no changes in the LDH activity in young toads. On the other hand, in older female (group III) the parameter was comparatively higher than that of older males, group II (Table 3 and Figure 1)

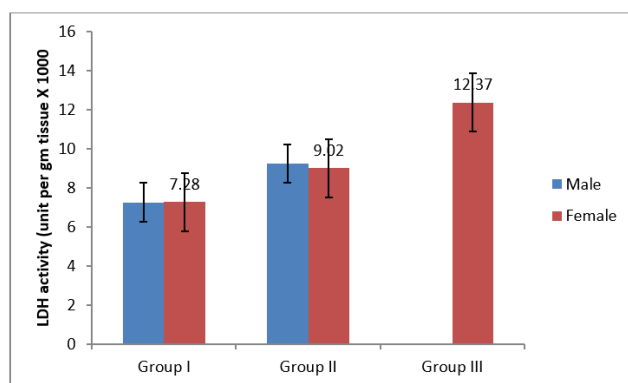


Fig. 1: Changes in Lactate dehydrogenase activity (Unit/g tissue x 1000) in liver of toad of different group in male and female

The lactate /pyruvate ratio in male toads decreases significantly ($p < 0.01$) in group II as compares to younger group I (-25%), whereas in female there was a significant

decrease in group II ($p < 0.01$) followed by an increase in group III ($p < 0.01$) (Table 7 and Figure 3). However the comparison between male and female indicated that Lactate/Pyruvate ratio in young and matured male toads were somehow having higher ratio than that of female. But the older females (group III) the parameters were comparatively in higher ratio than that of older males (group II).

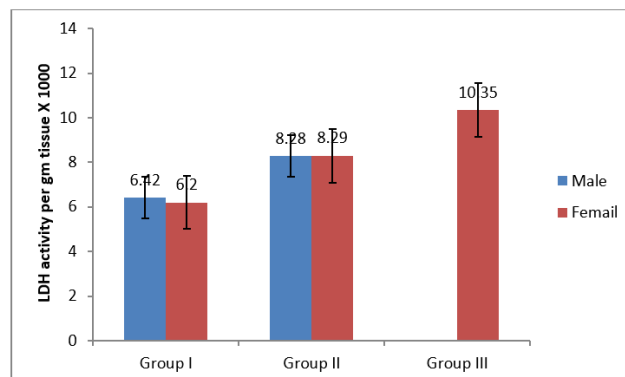


Fig. 2: Changes in Lactate dehydrogenase activity (Units/g tissue x1000) in skeletal muscle of toad of male and female in different group

3.3. Age related alterations in skeletal muscle

The age related change in lactate Dehydrogenase activity in skeletal muscle exhibited a significant increase ($p < 0.001$) in older group II male toads when compared to younger toad (group I). The parameter showed a significant increase in group II ($p < 0.001$) and group III female ($p < 0.01$). However, the comparison between male and female indicated that LDH activity in young male toads (group I) were having somehow higher activity that of younger female group I toads. On the other hand in adult females (group II) and in older females (group III) the parameter was comparatively with higher activity than that of older male group II toads (Table 5 and Figure 2).

There was no significant decrease of Lactate/pyruvate ratio in older male toads group II as compared to younger group I (-17.9%) where as in female there was a significant decrease in group II ($p < 0.001$) followed by significant increase in older females group III ($p < 0.001$) (Table 9 and Figure 4). However the comparison between male and female indicated that Lactate/pyruvate ratio in young female toads (group I) were having somehow higher ratio than that of younger male toads (group I). Also in the older females (group III) the parameter was comparatively in higher ratio than that of older males (group II).

Table 1: Changes in the S-V length (in cm) of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	6.65 + 0.27	N=19	<0.001	5.79 + 0.41	N=9	<0.001
Group II	8.4 + 0.13	N=12		8.48 + 0.19	N=10	
Group III				9.12 + 0.57	N=8	

Table 2: Changes in total liver weight (in gram) of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	0.654 + 0.063	19	<0.001	0.645 + 0.128	9	<0.001
Group II	1.717 + 0.59	12		1.721 + 0.103	10	
Group III				2.197 + 0.065	8	

Table 3: Changes in Lactate dehydrogenase activity (Unit/g tissue x 1000) in liver of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	7.26 + 0.33	19	<0.001	7.285 + 0.65	9	<0.001
Group II	9.25 + 0.06	12		9.02 + 0.25	10	
Group III				12.37 + 0.56	8	

Table 4: % Changes in Lactate dehydrogenase activity in liver

Among Groups	% Change
Male Group I vs Group II	27.3
Female Group I vs Group II	23.8
Female Group II vs Group III	45.9

Table 5: Changes in Lactate dehydrogenase activity (Units/g tissue x1000) in skeletal muscle of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	6.43 + 0.37	19	<0.001	6.2 + 0.69	9	<0.001
Group II	8.28 + 0.088	12		8.29 + 0.3	10	
Group III				10.35 + 0.59	8	

Table 6: % Changes in Lactate dehydrogenase activity of skeletal muscle

Among Groups	% Change
Male Group I vs Group II	28
Female Group I vs Group II	33.7
Female Group II vs Group III	24.8

Table 7: Changes in Lactate/Pyruvate ratio (ug/g tissue) in liver of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	7.2 + 0.59	19	<0.01	6.01 + 0.56	9	<0.01
Group II	5.4 + 0.57	12		3.82 + 0.26	10	
Group III				5.66 + 0.68	8	

Table 8: % Changes in Lactate/Pyruvate ratio (ug/g tissue) in liver of toad

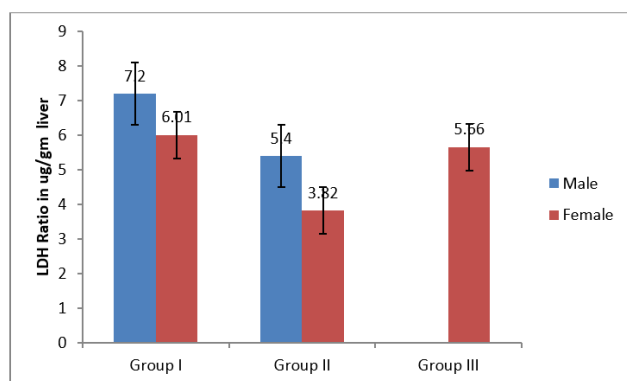
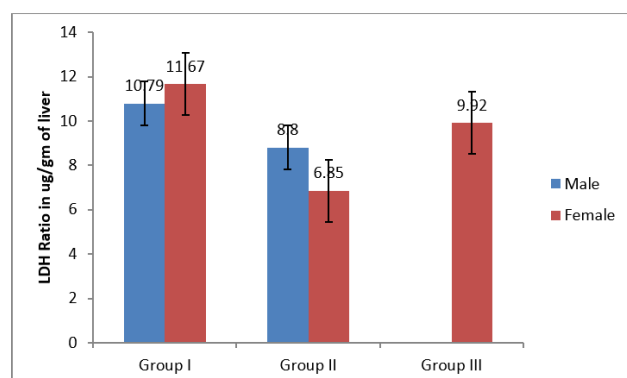
Among Groups	% Change
Male Group I vs Group II	-25
Female Group I vs Group II	-36.3
Female Group II vs Group III	+48

Table 9: Changes in Lactate/Pyruvate ratio (ug/g tissue) in skeletal muscle of toad

Age Groups	Male		P Value	Female		P Value
	Mean+ SEM	Sample size		Mean+ SEM	Sample size	
Group I	10.79 + 0.82	19	<0.01	11.67 + 1.38	9	<0.01
Group II	8.86 + 1.2			6.85 + 0.27	10	
Group III				8.9 + 0.38	8	

Table 10: Changes in Lactate/Pyruvate ratio (ug/g tissue) in skeletal muscle of toad

Among Groups	% Change
Male Group I vs Group II	-17.9
Female Group I vs Group II	-41.2
Female Group II vs Group III	+30.1

**Fig. 3:** Changes in Lactate/Pyruvate ratio (ug/g tissue) in liver of toad indifferent group of male and female**Fig. 4:** Changes in Lactate/Pyruvate ratio (ug/g tissue) in skeletal muscle of toad in different group of male and female

4. Discussion

Metabolic changes results to lead the imbalances in energy metabolism related to cellular and tissue homeostasis. Cellular lactate and pyruvate are key intermediates in intracellular energy metabolism in age-related phenomenon. During aging cellular lactate/pyruvate ratio taken to be a useful biomarker. The monitoring the key intermediate of cellular metabolic pathway through the lactate and pyruvate

are indicator of individuals energy conditions. Cellular level biochemical changes are associated with aging and age-related changes are of great importance to understand the state of health as well as disease. Unlike mammals and birds with determinant growth, poikilothermic vertebrates such as fishes, amphibians exhibit intermediate growth pattern.¹³ Reports on age related changes in LDH activities of tissues are inconsistent. However, in a majority of cases, an increasing in LDH activity with advancing age has been reported.¹⁴ Our results indicate that the hepatic LDH activity in both male and female common Indian toad increased with advancing age. The Lactate/Pyruvate ratio showed a decline during maturation in young to middle-aged group both in male and female toads and increased during middle aged to old. As liver and muscle are the master organ of body that determine the biochemical parameters of age related changes. Reports on age related changes in LDH activities decreased during aging which has been observed by many groups.^{15,16} However in some cases hepatic LDH activity increased with advancing age of male garden lizard *Calotes versicolor*.¹⁷ In this study, the hepatic LDH activity in both male and female increased with advanced age. It is likely that the liver of aging toads become more dependent on anaerobic glycolysis.

Aging process is highly complex due to cross-talk between various genetic signalling pathways, free radical production, mitochondrial dysfunction. Hence the unique ability of energy metabolic pathways during aging intimately related to the anaerobic and aerobic conditions during aging and health. Lactate and pyruvate promote stress resistance. Lactate is known to reduce the toxicity of different cellular insults.¹⁸ The answer is not yet clear, whether lactate support cell survival by boosting metabolism through increased mitochondrial respirations and ATP production or through changes in the NADH+/NAD+ redox ratio. In addition to the end product of glycolysis, lactate is considered as important energy substrate.¹⁹ Its role is not limited to energy production but also as signal for neuroprotection and synaptic plasticity. Lactate promotes a mild reactive oxygen species (ROS)

induction that translates into activation of antioxidant defences, which provides protection against oxidative stress. A mild induction of ROS by lactate promotes longevity through pro-survival pathway and pyruvate reduces the protective effects mediated by lactate.²⁰

5. Conclusion

Through this study, the finding accomplish that the hepatic LDH activity as well as LDH activity in skeletal muscle significantly increases with age in both the sexes. The hepatic lactate/pyruvate ratio declined in both sexes during maturation, however increased during later phase of life. Also in skeletal muscle, lactate/pyruvate ratio decreased significantly in advancing age but increased during later part of life in both sexes.

6. Limitations & Future Prospective

The limitations of this study is number of samples used and also higher vertebrate samples need to be compared. Future studies in higher mammals can be correlated with human being and the metabolic efficiency in liver and skeletal muscle will be of significant help in biomedical sciences.

7. Acknowledgement

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8. Source of Funding

None.

9. Conflict of Interest

None.

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