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Lactate dehydrogenase activity and isoenzyme patterns in the developing skeletal muscles of sialoadenectomised female mice

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Abstract

Lactate dehydrogenase (LDH) is essential for anaerobic glycolysis, catalyses the interconversion of lactate and pyruvate, critical for meeting rapid high energy demands and hence is necessary for muscular contraction in absence of oxygen. Objective of present study was to assess whether the polypeptides secreted by submandibular gland affect the physiology of developing skeletal muscles or not. For this study female mice at the age of twenty days were sialoadenectomised (removal of submandibular glands), when the submandibular gland starts the secretion of growth factors. The operated female mice were maintained under normal condition in the departmental animal house upto the age of 40 days, 60 days, 80 days and 100 days. Lactate dehydrogenase activity and electrophoretic separation of LDH from gastrocnemius, soleus and rectus abdominus muscles were determined in sialoadenectomised and control female mice in all four groups. Lactate dehydrogenase activity from all the three muscles was increased in sialoadenectomised mice as compared to control mice in all four groups. The electrophoretic separation of LDH also demonstrated the increase in intensity of LDH bands especially LDH-V in all the three muscles of sialoadenectomised mice. Thus, increase in LDH activity, especially LDH-V indicates anaerobic condition in muscle and also pathological conditions in the muscle due to sialoadenectomy.

INTRODUCTION

Lactate dehydrogenase (LDH) is an enzyme which catalyzes the reaction between pyruvate and lactate. LDH exists in five electrophoretically distinguishable isoenzyme forms that are tetramers of two different subunits, H and M, each under the control of a specific gene [1]. The M- subunit predominates in skeletal muscle and liver, the H- subunit predominates in heart muscle [2]. The LDH is important biomarker for pathological conditions inside the body.

Isoenzyme distribution patterns, particularly those of lactate dehydrogenase, have been extensively studied and are often tissue and species specific [3-6]. They are termed LD_1, LD_2, LD_3, LD_4 and LD_5. Lactate dehydrogenase (L-Lactate: NAD oxidoreductase, EC 1.1.1.27; LDH) plays an important role in regulation of anaerobic glycolysis through reoxidation of NADH [7, 8].

The submandibular gland of mouse secrete number of biologically active polypeptides like Epidermal growth factor (EGF), Nerve growth factor (NGF), Mesodermal growth factor (MGF) Glucagon, Kallikrein etc. Out of them MGF stimulates growth and differentiation of muscles and cartilages [9]. Atterdi in 1965 [9] studied effect of a fraction of submandibular gland of mouse on tissues of mesodermal origin in vitro. Later in 1967 [10], Atterdi tried to determine possible mode of action of submandibular gland extract on embryonic muscle tissue and showed that mesodermal growth factors from submandibular gland is responsible for proper differentiation of muscle tissue.

Previously Atterdi had tried to find out the possible role of submandibular MGF on mesodermal cells in the culture but previously nobody had tried to find out its role in *in vivo* conditions so the purpose of this investigation is to find out the role of salivary MGF on the LDH isoenzymes in the muscles. For this purpose an animal model was prepared in which submandibular gland was removed as they are rich source of MGF.

MATERIALS AND METHODS

Swiss albino female mice (*Mus musculus*) were used for present investigation. The mice were bred and reared in departmental animal house (Registration number- CPCSEA/ 233). The protocols of experiment were approved by animal ethics committee. They were housed in aluminum cages in groups of three to four and supplied with Amrut rat / mouse feed (Pranav Agro Industry) and water *ad Libitum*. For these study thirty two female mice of twenty days old weighing (8 to 15gms) were used for experimentation.

Sixteen female mice were sialoadenectomised at the age of 20 days and thereafter the sialoadenectomised mice were kept in animal house with proper conditions of light temperature, humidity and they were killed on 40^{th}, 60^{th}, 80^{th} and 100^{th} day of age from birth. Controls were sham operated. Mice from both groups were killed by decapitation and skeletal muscles viz., rectus abdominus, gastrocnemius and soleus were dissected out. The muscles were cleaned, weighed and homogenized in chilled distilled water. The concentration of homogenate was 5mg/ml for LDH estimation and 50mg/ml for electrophoresis. The homogenates were centrifuged at 10^{o}C at 5000 rpm for 10 minutes. The supernatants were used for estimation of lactate dehydrogenase by Sevela and Tovorek method (1989) [11] and electrophoresis of lactate dehydrogenase was carried out by using polyacrylamide gel electrophoresis [12-14].

STATISTICAL ANALYSIS

Results were interpreted with the help of one way analysis of variance for independent or correlated samples ANOVA followed by Tukey's post hoc test.

RESULTS

Lactate dehydrogenase activity in all the three muscles i.e. gastrocnemius, soleus and rectus abdominus muscle was increased significantly in sialoadenectomised female mice as compared to control mice from all groups i.e. from juvenile to

adult ($P < 0.001$) (Table no.1, 2 and 3). While the increase in the lactate dehydrogenase activity in all four groups from rectus abdominus muscle was more as compared to soleus and gastrocnemius muscle.

Plate no. A; Fig. no. 1, 2, 3 and 4 shows the electrophoretic separation of LDH from gastrocnemius muscle of 40 days, 60 days, 80 days and 100days old control and sialoadenectomised mice. The LDH in gastrocnemius muscle of all above groups in control mice was clearly separated into five bands i.e. LDH-I, II, III, IV and V, while in 40 days and 60 days aged sialoadenectomised mice all five bands were clearly separated and intensely stained than control and in 80 days and 100 days sialoadenectomised mice LDH was separated into five bands but the band IV was not clearly separated from V^{th} band and also very much intensely stained than the normal.

Plate no. B; Fig. no. 1, 2, 3 and 4 shows the electrophoretic

Table No.1 : Effect of sialoadenectomy on Lactate dehydrogenase activity on Gastrocnemius muscle of female mice (mMol/h/lit)

Groups	Control Female	Sialoadenectomised Female	Statistical significance
40 days (4)	2.5 ± 0.2915	4.64 ± 0.2408	P< 0.001
60 days (4)	4.44 ± 0.2702	7.18 ± 0.2387	P< 0.001
80 days (4)	5.46 + 0.4037	8.6 + 0.2236	P< 0.001
100 days (4)	7.1 ± 0.114	11.0 ± 0.1304	P< 0.001

Number in parenthesis denotes the number of animals.

Table No.2 : Effect of sialoadenectomy on Lactate dehydrogenase activity on Soleus muscle of female mice (mMol/h/lit)

Groups	Control Female	Sialoadenectomised Female	Statistical significance
40 days (4)	1.63 ± 0.0365	3.28 ± 0.2775	P< 0.001
60 days (4)	4.402 ± 0.396	6.14 ± 0.2302	P< 0.001
80 days (4)	6.1 + 0.1924	8.6 + 0.3114	P< 0.001
100 days (4)	7.132 ± 0.0858	11.0 ± 0.3317	P< 0.001

Number in parenthesis denotes the number of animals.

Table No.3 : Effect of sialoadenectomy on Lactate dehydrogenase activity on Rectus abdominus muscle of female mice (mMol/h/lit)

Groups	Control Female	Sialoadenectomised Female	Statistical significance
40 days (4)	2.68 ± 0.023	6.2 ± 0.1924	P< 0.001
60 days (4)	4.74 ± 0.1924	8.40 ± 0.2302	P< 0.001
80 days (4)	6.0 + 0.0912	9.66 + 0.4278	P< 0.001
100 days (4)	8.38 ± 0.0676	12.3 ± 0.1924	P< 0.001

Number in parenthesis denotes the number of animals.

Plate no. A Electrophoretic Separation of LDH from Gastrocnemius muscle of Control and Sialoadenectomised Female mice

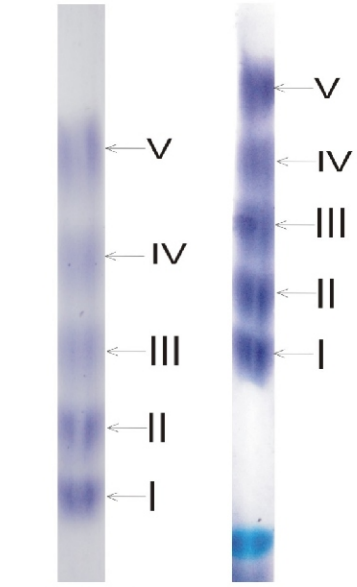


Figure no.1

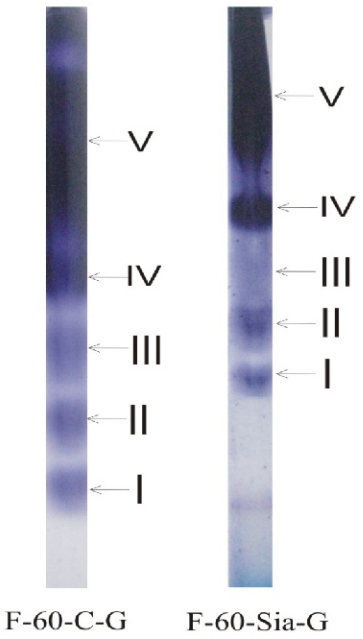


Figure no.2

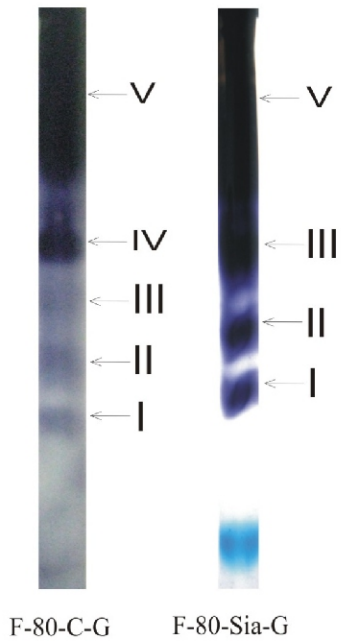


Figure no.3

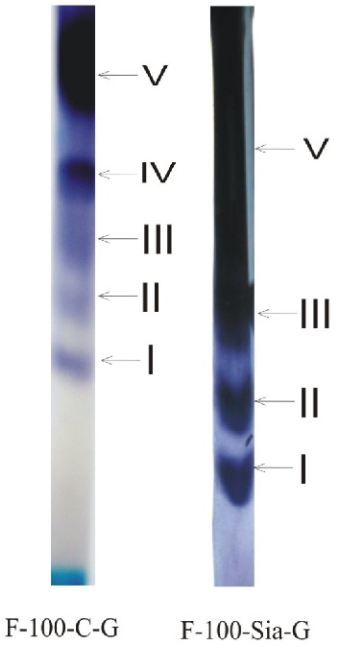


Figure no.4

Plate No. A: Electrophoretic separation of LDH from Gastrocnemius muscle of control and sialoadenectomised female mice.

Figure No. 1, 2, 3, 4 indicates-	F- Female mice	40, 60, 80 and 100- Age of animals
C- Control mice	Sia- Sialoadenectomised mice	G- Gastrocnemius muscle

Plate No. B Electrophoretic Separation of LDH from Soleus muscle of Control and Sialoadenectomised Female mice

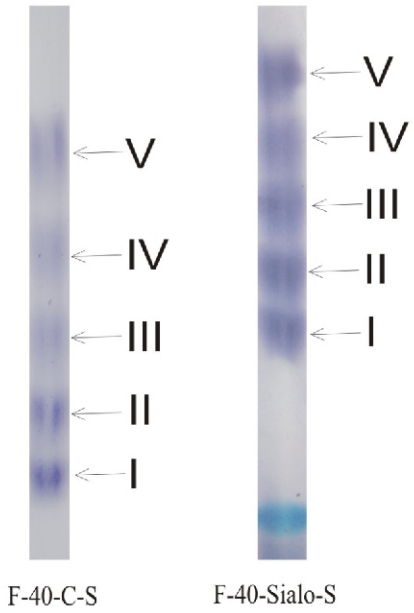


Figure no. 1

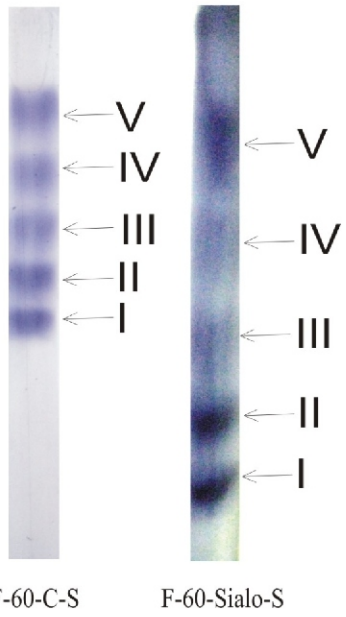


Figure no. 2

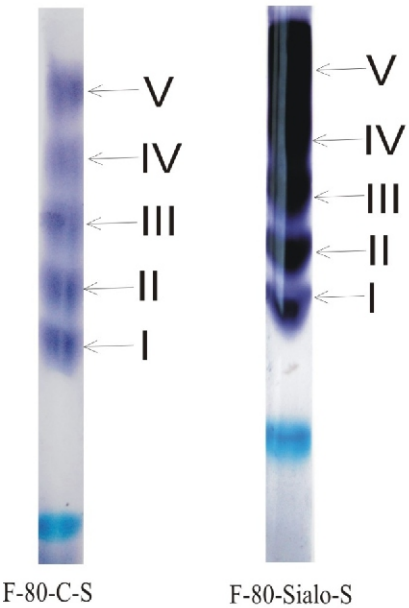


Figure no. 3

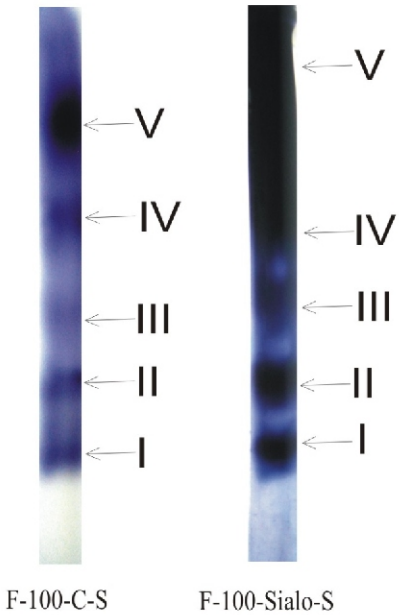


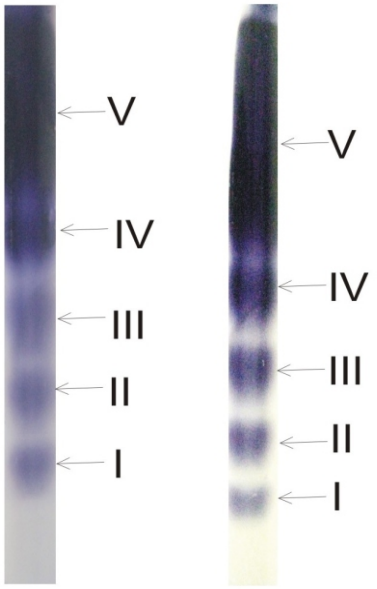
Figure no. 4

Plate No. B: Electrophoretic separation of LDH from Soleus muscle of control and sialoadenectomised female mice.

Figure No. 1, 2, 3, 4 indicates- F- Female mice 40, 60, 80 and 100- Age of animals

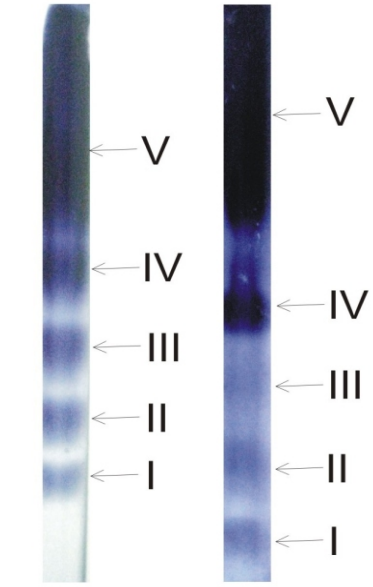
C- Control mice Sia- Sialoadenectomised mice S- Soleus muscle

Plate No. C Electrophoretic Separation of LDH from Rectus abdominus muscle of Control and Sialoadenectomised Female mice



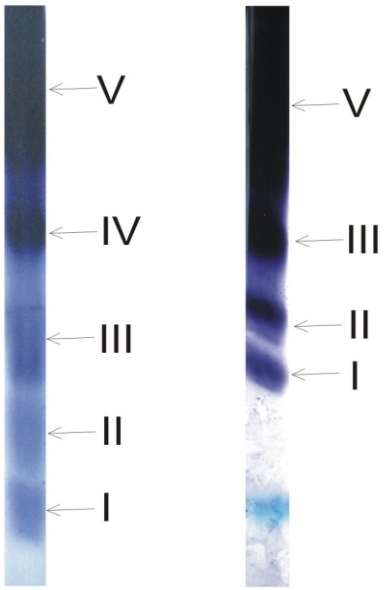
F-40-C-R F-40-Sia-R

Figure no. 1



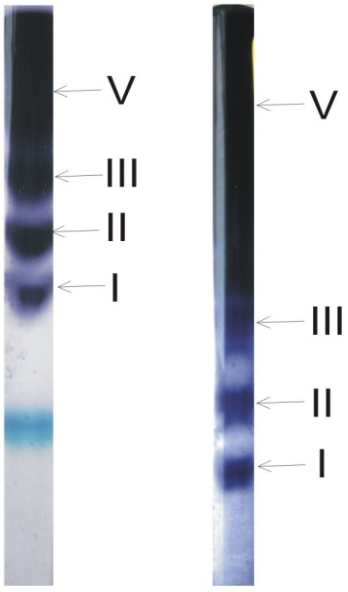
F-60-C-R F-60-Sia-R

Figure no. 2



F-80-C-R F-80-Sia-R

Figure no. 3



F-100-C-R F-100-Sia-R

Figure no. 4

Plate No. C: Electrophoretic separation of LDH from Rectus abdominus muscle of control and sialoadenectomised female mice.

Figure No. 1, 2, 3, 4 indicates- F- Female mice 40, 60, 80 and 100- Age of animals

C- Control mice Sia- Sialoadenectomised mice R- Rectus abdominus muscle

separation of LDH from soleus muscle of 40 days, 60 days, 80 days and 100 days old control and sialoadenectomised mice. The LDH in soleus muscle of all above groups were clearly separated into five bands in control mice. While in 40 days sialoadenectomised mice all bands were clearly separated and were intensely stained as compared to control, whereas in 60 days sialoadenectomised mice the I, II and V band were intensely stained than control and in 80 days and 100 days sialoadenectomised mice I and II band was intensely stain and IV and V band were not clearly separated from each other and were much intensely stained than control.

Plate no. C; Fig. no. 1, 2, 3 and 4 shows the electrophoretic separation of lactate dehydrogenase from rectus abdominus muscle of 40 days, 60 days, 80 days and 100days old control and sialoadenectomised mice. The LDH in rectus abdominus muscle of 40, 60, and 80 days old control mice was clearly separated into five bands but in 100 days old mice the IV and V band was not clearly separated from each other. While in 40 days old sialoadenectomised mice the III, IV and V bands and in 60 days old sialoadenectomised mice the IV and V bands were intensely stained than the normal, whereas in 80 days and 100 days old sialoadenectomised mice the III, IV and V bands were intensely stained than control and IV band was not clearly separated from V band.

DISCUSSION

Lactate dehydrogenase activity in all the three types of skeletal muscle, in 40 days, 60 days, 80 days and 100 days old female mice was increased significantly in sialoadenectomised mice as compared to control mice.

Lactate dehydrogenase is essential for anaerobic glycolysis and hence is necessary for muscular work in absence of oxygen. LDH is an enzyme that under anaerobic conditions catalyzes the reversible transformation of pyruvate to lactate. Upregulation of LDH ensures an efficient anaerobic/ glycolytic metabolism for tumor cells and reduced dependence on oxygen [15].

As the number of the M over H chains increases the LDH isoenzymes become more efficient in catalyzing the conversion of pyruvate to lactate (LDH-V), whereas an increase of H over M chains (LDH-I) favours the conversion of pyruvate to acetyl CoA that enters into the citric acid. The total concentrations of LDH and the proportions of the five isoenzymes relative to one another have been found to vary from tissue to tissue within the same individual and it also vary during development within a given tissue which is reflected due to differential gene action.

In medical research changes of LDH pattern in serum has been employed for detection of pathophysiological changes in the organism [16-19]. The isoenzymes differ in their catalytic activity, for LDH-I is inhibited by lower concentrations of pyruvate than is LDH-V [20-24]. After sialoadenectomy there was increase in staining intensity of LDH bands especially LDH-V. Under conditions of low oxygen tension LDH-V would be expected to predominate over LDH- I. The LDH-V is more efficient in catalyzing the conversion of pyruvate to lactate. This indicates that after sialoadenectomy the muscle tend to be more equipped for anaerobic type of metabolism.

Thus, increase in LDH activity especially LDH-V indicates anaerobic condition in muscle and also pathological conditions in the muscle due to absence of submandibular gland secreted MGF.

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