Eur J Clin Chem Clin Biochem 1996; 34:961–964

Age-Dependent Variations of Lactate Dehydrogenase and Creatine Kinase Activities in Water Buffalo Calf Serum

Luigi Avallone[1], *Pietro Lombardi*[1], *Salvatore Florio*[1], *Antonio d'Angelo*[1] and *Eitan Bogin*[2]

[1] Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università degli Studi di Napoli Federico II, Napoli, Italy

[2] Department Biochemistry, Kimron Veterinary Institute, Tel Aviv University School of Medicine, Beit Dagan, Israel

Summary: The electrophoretic patterns of the serum enzymes lactate dehydrogenase and creatine kinase from water buffalo calves are described. Differences in total activities as well as their relative distribution were seen at ages ranging from 1 to 10 weeks. While total lactate dehydrogenase activity increased by over 100%, total creatine kinase increased by almost 400%. The relative activities of lactate dehydrogenase 1 and 5 decreased with age. Lactate dehydrogenase 2 and 3 increased and lactate dehydrogenase 4 did not change.

In relation to creatine kinase, the prevalent isoenzyme was creatine kinase-MM, but it's relative activity gradually decreased in comparison to the other two isoenzymes (creatine kinase-MB and creatine kinase-BB). Creatine kinase-BB was completely absent until the 3rd week of age. The percentage modifications of creatine kinase isoenzymes were correlated to age.

The results suggest that isoenzymatic separation and characterization of lactate dehydrogenase and creatine kinase in relation to the various tissues can significantly contribute to the diagnosis of diseases which are linked to tissue damage.

Introduction

Isoenzymes are multiple enzyme forms, having a similar catalytic activity and different physical characteristics (1). The enzymes lactate dehydrogenase and creatine kinase are hybrid isoenzymes, with aggregations of different subunits of two or more proteins (1, 2).

Quantification of these enzymes is important in the diagnosis of diseases, especially at the neonatal stage of life, a period characterized with numerous diseases (3–7). The elucidation of the normal reference values of the two enzymes and their isoenzyme patterns at the neonatal stage of life can be important for the better diagnosis and understanding of diseases. Having a detailed and complete picture of the metabolic and health condition of the calf is important because of the many neonatal diseases occurring at this state of life.

The objective of the present communication is to determine reference values of lactate dehydrogenase and creatine kinase and the isoenzyme patterns in 1–10 week old buffalo calves.

Materials and Methods

Specimens

Six water buffalo calves (weaning) were used. The calves were fed ad libitum with fresh whole maternal milk taken from the mothers. Blood was withdrawn from the jugular vein every week for 10 weeks, allowed to clot and serum was obtained by centrifugation at 600 *g* for 15 min. Blood was always taken at the same time of day to avoid possible circadian variation and was measured within 24 hours of its withdrawal.

Enzymes

Lactate dehydrogenase (lactate : NAD^+ oxidoreductase EC 1.1.1.27) and creatine kinase (ATP : creatine N-phosphotransferase EC 2.7.3.2) were measured spectrophotometrically at 37 °C (8, 9). The measurements were done using a Perkin-Elmer spectrophotometer. Isoenzyme electrophoresis was performed using Hydrogel iso-LDH and iso-CK kits (Sebia, France) (10). Following staining, the relative activity of each isoenzyme fraction was determined using a densitometer (Ciampolini, Italy). Internal quality control was used (Precinorm-4 and Precipath-4, Boehringer Mannheim, Germany).

Statistics

Means, standard deviation and significance were determined using the general linear model (GLM) procedure of the SAS program (11).

Results

The absolute levels of lactate dehydrogenase and the relative distribution of the isoenzymes are shown in table 1. There was a slow and gradual increase in total lactate dehydrogenase activity from mean values of 1314 (U/l) at week 1 to 2208 (U/l) at week 10. At week 2, a sharp

Tab. I Total and relative activities of lactate dehydrogenase isoenzymes in buffalo calf serum (mean ± SD; n = 6)

Age (weeks)	Total activity (U/l)	Isoenzyme 1		Isoenzyme 2		Isoenzyme 3		Isoenzyme 4		Isoenzyme 5	
		U/l	%	U/l	%	U/l	%	U/l	%	U/l	%
1	1314 ± 225	766 ± 179	58.4 ± 7.2	264 ± 79	20.1 ± 3.1	158 ± 92	11.9 ± 3.6	70 ± 21	5.3 ± 0.7	57 ± 46	4.3 ± 1.7
2	995 ± 93	652 ± 50	65.5 ± 5.3	184 ± 21	18.5 ± 2.2	93 ± 11*	9.3 ± 1.1	38 ± 19*	3.8 ± 2.2	28 ± 17*	2.8 ± 1.7
3	1205 ± 155	703 ± 20	58.3 ± 1.3	283 ± 17	23.5 ± 1.1	133 ± 25*	11.0 ± 1.5	52 ± 12*	4.3 ± 0.7	22 ± 22*	1.8 ± 1.4*
4	1235 ± 299	689 ± 9	55.8 ± 0.2	324 ± 42*	26.2 ± 1.4*	201 ± 45*	16.6 ± 1.5	31 ± 21*	2.5 ± 0.7	22 ± 24*	1.8 ± 0.8*
5	1420 ± 173	796 ± 43	56.1 ± 2.4	379 ± 38*	26.7 ± 2.1*	185 ± 42*	13.0 ± 7.4	41 ± 31*	2.9 ± 1.8	23 ± 12*	1.5 ± 0.7*
6	1430 ± 87	758 ± 27	53.1 ± 3.1	398 ± 31*	27.8 ± 3.5*	182 ± 24*	12.6 ± 2.7	57 ± 10*	4.1 ± 1.1	36 ± 13*	2.4 ± 1.4
7	1893 ± 149	1001 ± 25	52.9 ± 1.6	534 ± 40*	28.1 ± 3.0*	254 ± 48*	13.4 ± 3.2	70 ± 18	3.7 ± 1.1	36 ± 15*	1.9 ± 0.9*
8	2100 ± 191	1098 ± 77	52.3 ± 4.1	588 ± 32*	28.1 ± 1.8*	288 ± 59*	13.7 ± 3.1	88 ± 15*	4.2 ± 0.7	29 ± 13*	1.4 ± 0.7*
9	2174 ± 173	1131 ± 47	52.9 ± 2.1	602 ± 31*	27.7 ± 1.7*	300 ± 29*	13.8 ± 1.7	87 ± 12*	3.9 ± 0.6	35 ± 11*	1.6 ± 0.5*
10	2202 ± 157	1009 ± 85	45.8 ± 5.1*	696 ± 23*	31.6 ± 1.5*	355 ± 42*	16.1 ± 2.7	104 ± 42*	4.7 ± 2.6	40 ± 22*	1.8 ± 1.3*

* Significantly different ($P < 0.05$) from week 1

decrease of about 24% was seen, reaching mean values of 995 (U/l) and gradually increasing thereafter. Lactate dehydrogenase 1 was the major isoenzyme, consisting of 58.4% of the total activity at week 1. Even though absolute activity increased by 10%, the relative activity decreased to 45.8%. Lactate dehydrogenase 2, the second largest fractions, was 20.1% of the total activity at week 1, and increased by 131% at week 10, to 695 U/l, but only by 10.5% in its relative activity. Lactate dehydrogenase 3 increased from a mean of

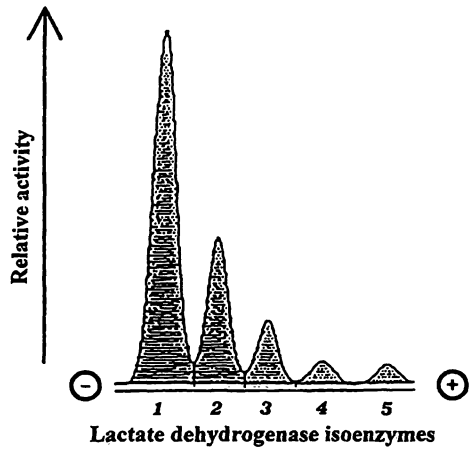

Fig. 1 Distribution of serum lactate dehydrogenase isoenzymes in 3 week old calves (representative example).

Lactate dehydrogenase isoenzyme

Isoenzyme	Fraction of total activity (%)
1	58.2
2	22.9
3	9.5
4	5.5
5	3.9

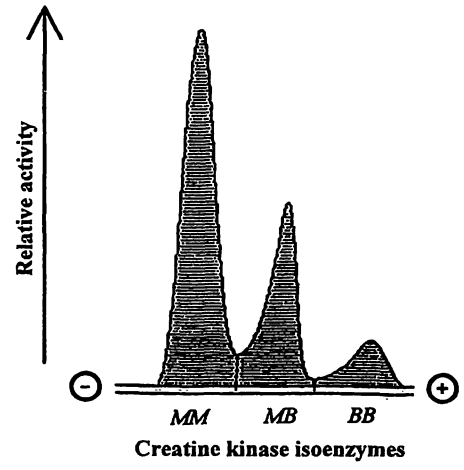

Fig. 2 Distribution of serum creatine kinase isoenzymes in 5 week old calves (representative example).

Creatine kinase isoenzymes

Isoenzyme	Fraction of total activity (%)
MM	61.6
MB	28.5
BB	9.9

Tab. 2 Total and relative activities of creatine kinase isoenzymes in buffalo calf serum (means ± SD; n = 6)

Age (weeks)	Total activity (U/l)	Isoenzyme-MM		Isoenzyme-MB		Isoenzyme-BB	
		U/l	%	U/l	%	U/l	%
1	130 ± 27	123 ± 30	94.2 ± 0.2	8 ± 4	5.8 ± 1.5	–	–
2	246 ± 30	216 ± 28*	87.6 ± 9.5	31 ± 9.2*	12.4 ± 3.2	–	–
3	380 ± 79	335 ± 66*	88.1 ± 8.4	45 ± 22*	11.9 ± 2.8	–	–
4	346 ± 108	266 ± 332*	76.8 ± 9.6*	65 ± 38*	18.7 ± 3.5	16 ± 10*	4.5 ± 0.9*
5	312 ± 48	204 ±34*	65.4 ± 7.2*	70 ± 23*	22.3 ± 4.8	38 ± 8*	12.3 ± 1.8*
6	281 ± 33	175 ± 27*	62.1 ± 8.1*	55 ± 27*	19.7 ± 3.2	51 ± 7*	18.2 ± 2.1*
7	428 ± 236	256 ± 193*	59.7 ± 8.2*	92 ± 77*	21.4 ± 3.0	81 ± 54*	18.9 ± 2.3*
8	553 ± 104	300 ± 65*	54.3 ± 6.3*	137 ± 43*	24.7 ± 4.2	116 ± 3*	21.0 ± 2.6*
9	549 ± 359	307 ± 194*	55.8 ± 5.4*	152 ± 10*	27.6 ± 2.9	91 ± 6*	16.6 ± 1.7*
10	511 ± 322	253 ± 18*	49.5 ± 5.6*	170 ± 12*	33.3 ± 3.7	88 ± 11*	17.2 ± 3.5*

* Significantly different ($P < 0.05$) from week 1

157.7 U/l and 11.9% relative activity at week 1, to a mean of 354.5 U/l at week 10, an absolute increase of 125%, but only 4.2% in the relative activity. Lactate dehydrogenase 4, showing absolute activity of 70 U/l and 5.3 ± 0.7% relative activity at week 1, increased by 53% in absolute activity, but almost none in its relative activity. Lactate dehydrogenase 5 was the only enzyme showing a decrease, from 57 at week 1 to 40 U/l (30%) at week 10. The decrease was more dramatic when evaluated as relative activity, from 4.3 ± 1.7% to 1.8 ± 1.3%, a decrease of almost 60%.

The absolute serum levels of creatine kinase and relative distribution of the isoenzymes during the first 10 weeks of life are given in table 2. A gradual increase in the absolute levels of the enzymes is seen with the growth of the animals from a mean of 130 to 511 U/l, with a mean increase of almost 300%. The increased activity of creatine kinase during the first 10 weeks of life was attributed to the significantly increased activities of creatine kinase-MB and creatine kinase-BB, which were negligible at birth, reaching levels of about 50% of the total creatine kinase activity. Representative examples of lactate dehydrogenase and creatine kinase electrophoretically separated isoenzymes are shown in figures 1 and 2 and the ratio between them during the first 10 weeks of their lives are given in figure 3. As seen, while the levels of enzymes, both total and relative, increased during this period, the ratio between lactate dehydrogenase 1 and lactate dehydrogenase 2 decreased.

Discussion

The level of many enzymes in the blood of man and animals are determined by

(a) rate of release into the blood stream,
(b) rate of removal from the blood stream.

Two enzymes, lactate dehydrogenase and creatine kinase and their isoenzymes were evaluated in buffalo calves blood during the first 10 weeks of their lives, demonstrating the major and dynamic changes which take place in the blood as a reaction to the physiological changes occurring during growing and development.

During this time some major changes take place in the metabolism of the newborn calf. These include an early stage adaptation to the newborn less protective and more independent life (3, 4), development of the digestive system including the rumen and of new metabolism (5), development of skeletal muscle (12), rapid growth rate and more. As a result of the above, the contribution of each of these systems varies according to the activities of these tissues and consequently may lead to a changing blood picture.

Establishing normal reference values is of great importance to each animal species at each stage of life. Having a defined range for each age and other subgrouping will enable a better and more accurate diagnosis of the abnormal and pathological conditions.

The use of electrophoresis for the identification of the isoenzyme distribution can further contribute to a more refined resolution of changes seen and the possible origin of the contributing tissues or the damaged organs.

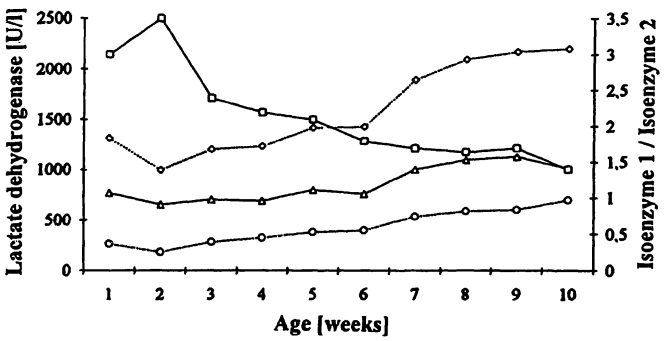

Fig. 3 Levels of total lactate dehydrogenase, its isoenzymes 1 and 2 and their ratio in the serum of 1–10 weeks old buffalo calves.

Lactate dehydrogenase
···◇··· Total
—△— Isoenzyme 1
---○--- Isoenzyme 2
—□— Isoenzyme 1/Isoenzyme 2

The present communication describes some new important clinical information, a great use in the growing field of buffalo production and a critical age during a time of high frequency of neonatal diseases.

The results obtained show that buffalo species have isoenzyme patterns similar to those seen in man and other animals. The lactate dehydrogenase is a tetramer having five isoenzymes, with a domination of lactate dehydrogenase 1 then followed by 2 and 3 and finally about the same amounts 4 and 5.

The serum creatine kinase is a dimer with three isoenzymes, dominated by the MM and followed by MB and BB.

The data show the gradual changes in the absolute serum levels of lactate dehydrogenase doubling its value within 10 weeks. This dramatic increase in enzyme activity is probably a result of enzyme contribution from other sources such as liver, kidney, heart etc. This is supported from the fact that the changes in the relative and absolute contribution of each isoenzyme of lactate dehydrogenase to the pool is different in trends and amounts. A similar picture but even more dramatic is the absolute levels and the relative contribution of the isoenzymes.

Creatine kinase MM increased only by about 100% from week 1 to 10, the isoenzyme MB increased during this period by over 2000% and the isoenzyme BB, which was not detectable at week one consisted of about 18% of total creatine kinase activity, equivalent to 88 U/l, suggesting the contribution of non-skeletal tissue having a different isoenzyme pattern to the creatine kinase pool in the serum.

The data presented show the buffalo calf serum to resemble the one in cattle, showing similar values of the lactate dehydrogenase and creatine kinase levels as well as the relative distribution of their isoenzymes (4, 13).

It is of interest that at week 2, a significant drop in lactate dehydrogenase levels were seen, gradually increasing thereafter. It is possible that the drop seen is after a large increase caused by colostral ingestion, immediately after birth and the entrance of the colostral lactate dehydrogenase to the calf serum. It was shown (4) that colostral enzymes ingested within the first day of life enter the blood serum, maintain their catalytic activity and slowly clear out.

It is therefore possible to postulate that in the calf, two sources of enzymes enter the blood, one from the colostrum, which is fast, and short-lived, and the second, which originates from the various tissues and is slow and continuous. At week 2, following the clearance of the colostral enzyme, a lower value was seen. This hypothesis is supported by the fact that no similar pattern was seen with the enzyme creatine kinase. This was due to the fact that colostrum and milk contain little or no creatine kinase but contain high levels of lactate dehydrogenase (4, 13).

The change of the isoenzyme profile and the absolute levels of each one, shed light on the development changes which take place with growth during the first 10 weeks. It shows the potential of electrophoresis of isoenzymes in the elucidation of changes and their possible relationship to the various organs and metabolism.

The lactate dehydrogenase ratio of 1 to 2 calculated and depicted in figure 3, can serve as an indicator of the stage of the calfs development reaching the point of steady state metabolism, which is at around a ratio of 1.0.

References

1. Lehninger AL. Biochemistry. 2nd ed. New York, USA: Worth Publishing, 1975.
2. Tietz N. Fundamentals of clinical chemistry. Philadelphia, USA: WB Saunders Company, 1986.
3. Bogin E. Handbook for veterinary clinical chemistry E. New York, USA: Kodak Publ 1992.
4. Bogin E, Avidar Y, Shenkler S, Israeli BA, Spiegel N, Cohen R. A rapid field test for the determination of colostral ingestion by calves based on γ-glutamyltransferase. Eur J Clin Chem Blin Biochem 1993; 31:695–9.
5. Yancik SA, McIlwraith LW, Wangear AE, Trotter GW. Evaluation of creatine kinase and lactate dehydrogenase activities in clinically normal and abnormal equine joints. J Vet Res 1987; 48:463–6.
6. Hatzipanagitou A, Lindner A, Sommer H. Lactic dehydrogenase and creatine kinase isoenzyme patterns in the blood plasma of horses with elevated creatine kinase, lactic dehydrogenase and aspartate amino-transferase activities. Dtsch Tierärztl Wochenschr 1991; 98:284–6.
7. Michalek A, Markanik J. Activity values of lactate dehydrogenase isoenzymes in the serum of farm animals. Vet Med 1975; 20:199–206.
8. Hørder M, Magid E, PitKänen E, HarKonen M, Strømme JH, Theodorsen LV, et al. Recommended method for the determination of creatine kinase in blood modified by the inclusion of EDTA. Scand J Clin Lab Invest 1979; 39:1–5.
9. Keiding R, Hørder M, Gerhardt W, PitKäne E, Tehunen R, Strømme JH, et al. Recommended methods for the determination of four enzymes in blood. Scand J Clin Lab Invest 1974; 33:291–306.
10. Abbot LB, Van Lente F. Procedure for the characterization of creatine kinase on agarose electropherograms. Clin Chem 1985; 31(3):445–7.
11. SAS Institute Inc. SAS user's guide: statistics. SAS Institute Inc. Carry, NC, NY, 1985.
12. Kanemitsu F, Okigaki T. Creatine kinase isoenzymes. J Chromatogr 1988; 429:399–417.
13. Kaneko JJ. Clinical biochemistry of domestic animals. London, Toronto, San Francisco: Acad Press 1989.

Received June 17/September 26, 1996

Corresponding author: Professor Dr. Eitan Bogin, Department of Biochemistry, Kimron Veterinary Institute, Tel Aviv University School of Medicine, P.O. Box 12, Beit-Dagan 50250, Israel