Effect of Dexamethasone Stress on Concentrations of Zinc in Blood Plasma and in Sub-Cellular Fractions of Various Tissues of Neonatal Buffalo Calves

Charanbir Singh* and S. P. S. Singha

Department of Veterinary Biochemistry, College of Veterinary Science, Punjab Agricultural University Ludhiana-Punjab, India

ABSTRACT : Effect of chemical stress of daily administration of glucocorticoid (dexamethasone @0.125 mg./calf/day) injections on plasma zinc levels, Zn status of body tissues and its distribution in sub cellular fractions, was studied in neonatal buffalo calves. Daily i/m injections of dexamethasone, starting at the completion of 1 week of age and continued till 8th week, led to a significant decline in plasma Zn concentration from 3rd week onwards, which then persisted throughout the rest of the experimental period. In control group, liver had the highest concentration of zinc, followed by heart, muscle, spleen, kidney and testis. In all these tissues, cytosolic fractions had the highest (>60%) zinc levels followed by nuclear, mitochondrial and microsomal fractions. In dexamethasone treated calves, there was a significant increase in the Zn uptake by the tissues of liver and muscle. This increase in zinc concentration was observed in all the sub cellular fractions of liver and muscle, however about 80% of this increase was in cytosolic fraction. It was concluded that glucocorticoid-induced stress caused increase in Zn levels of liver/muscles and decrease in blood plasma zinc, thus indicating a redistribution of Zn in body. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 1022-1025)***

Key Words : Dexamethasone, Zinc Status, Plasma, Tissues, Neonatal Buffalo Calves

INTRODUCTION

Zinc is an essential nutrient for animals and plays an indispensable role in a number of body functions. It is widely distributed throughout the body and about 99% of the total body zinc is intracellular . Many factors can affect the concentration of zinc in blood besides the dietary Zn intake, among these are, acute phase of many disease processes (Falchuk, 1977), infections like mastitis (Wegner et al., 1973) and during various forms of stressing conditions like administration of hormones like corticosteroids (Flynn et al., 1971) and hyper-thermal stress. Most of these studies were carried either in humans or in adult dairy cattle and the information is scanty about the changes in zinc status in young calves subjected to stressing conditions. This experiment was planned to monitor changes in the Zn status of plasma, distribution of zinc in various tissues and in sub cellular fractions in neonatal buffalo calves which were subjected to the stress of daily administration of glucocorticoid (Dexamethasone) injections.

MATERIALS AND METHODS

New born male buffalo calves used for the present study were procured from dairy farm, PAU, Ludhiana and housed in well ventilated, concrete floored calf pens. These calves were milk fed @3.5 kg per calf per day. For this purpose, fresh buffalo milk was obtained daily in the morning and evening and pail fed immediately. Calves were maintained on whole milk diet during the experimental period. Drinking water was available round the clock.

Calves were divided into two groups of five each. Intramuscular injections of 0.9% normal saline and dexamethasone (0.125 mg in 1 ml. of 0.9% normal saline) were administered daily in the morning to the calves of control and dexamethasone group respectively. The treatment was started on the completion of 1 week of neonatal age and continued till the calves attained an age of 8 weeks. Dexona (dexamethasone) M/s Cadila Pharmaceuticals, India was procured from the market.

Blood samples were collected weekly in heparinized and dried collection vials under aseptic conditions. After 8 weeks, the buffalo calves were euthanized by bleeding through the common carotid artery. Tissue samples of liver, kidney, spleen, heart, muscle and testis were collected immediately thereafter. For sub-cellular fractionation of tissues, all procedures were carried out at 0 to 4°C. Duplicate samples (2.5 g) of each tissue were homogenized with 6 volumes of ice cold 0.25 M sucrose in a tissue homogenizer fitted with teflon pestle. The pestle was driven at approximately 1,000 rpm for five up and down strokes of about 5 seconds each. Sub-cellular fractionation was performed as described by Porter et al. (1961). The homogenates were centrifuged at 600×g for 10 minutes for nuclear and at 8,500×g for 12 minutes mitochondrial fraction. The resulting supernatants were centrifuged at 105,000×g for 60 minutes to separate microsomal fraction from the cytosolic fraction.

* Corresponding Author: Charanbir Singh.

Received November 7, 2001; Accepted February 18, 2002

Zinc status of plasma, whole tissues and in sub-cellular fractions of various tissues was analyzed by the method of Ludmilla. (1976). Each sample was digested in nitric, perchloric and sulfuric acid (10:3:1.v/v). Using standards prepared in the same matrix as the digested samples. Zn determination was done by Atomic absorption spectrophotometer (Varian Techtron AA6. Australia).

Weekly means. standard error (S.E) values. comparison between means (Student's 't' test) were carried out by digital calculator. For statistical work. methods detailed by Snedecor and Cochran (1976) were followed.

RESULTS AND DISCUSSION

Plasma zinc concentration

In control group. from a low level in Ist week of life, zinc concentration increased rapidly during 2nd and 3rd week and thereafter declined steadily (figure 1) to a concentration of 16.37 μmol/L at 8th week of life. But for higher values observed during 3rd and 4th week. plasma Zn levels recorded in the present study are in agreement with the reported (Miller et al., 1971; Sawadogo et al., 1988; Carcagno et al., 1994) plasma zinc concentration of 16.9-17.5 μmol/L for young growing calves.

In dexamethazone treated group. the weekly plasma zinc concentrations were significantly lower than the control group averages at weeks 3rd and 4th ($p<0.05$) and at 5th. 7th and 8th ($p<0.01$). A continuous downward trend in plasma Zn level was observed from 3rd week onwards (figure 1). Percentage decline in Zn concentration from that of control group was 1.9%. 17.1%, 22.4%. 27.7%. 18.9%, 39.5% and 33.9% during 2nd. 3rd. 4th. 5th. 6th. 7th and 8th weeks respectively.

Lower plasma Zn concentrations in dexamethasone treated calves are in accordance with the findings of Flynn et al. (1971). Henkin (1974). Falchuk (1977) and Vallee and Falchuk (1993). They reported that corticosteroids lowered plasma zinc by 30% to 40%. which persisted as long as the hormone continued to be administered. Weeks et al. (1989) also reported a gradual fall in serum zinc in cattle receiving i/v dexamethasone (2 mg) for 3 days. These results. however, do not agree with the observations of Wegner et al (1973) who recorded that simulation of a stressing condition by a single injection of corticotropin did not affect serum zinc and even extending the duration of stress by giving four consecutive daily injections of corticotropin did not statistically significantly change the serum zinc during 72 h injection period. But their study was conducted on 18 months old or lactating cows. which were administered 1 or 4 corticotropin injections. Therefore varying results in our experiment might be due to lesser age of animals and longer duration of stress because neonatal calves were subjected to dexamethasone injections daily till 8 weeks of age. Thus duration of the stressing condition may be an important factor in the stress zinc relationship because statistically significant change in plasma Zn appeared only at 3rd week in our experiment.

Zinc concentration in tissues and sub cellular fractions

Mean values of Zn concentration of various tissues and in sub cellular fractions are presented in table 1.

In control group, highest zinc concentration (figure 2) was observed in liver followed by heart. muscle. spleen. kidney and testis. Zinc concentration in sub cellular

Table 1. Mean zinc concentration (μg/g wet tissue). in various tissues and their subcellular fractions following daily i/m administration of dexamethasone

Tissues	Control group					Dexamethazone group				
	Whole tissue	N.F	M.F	Mc.F	C.F	Whole tissue	N.F.	M.F	Mc.F	C.F
Liver	33.25 ±1.97	8.67 ±0.68	1.66 ±0.22	0.42 ±0.07	20.93 ±1.22	44.54* ±1.19	9.94 ±0.60	2.11 ±0.38	0.48 ±0.04	29.99* ±1.78
Kidney	20.65 ±1.22	4.47 ±0.28	0.77 ±0.17	0.47 ±0.05	13.87 ±0.80	21.03 ±1.66	5.19 ±0.40	1.50 ±0.24	0.43 ±0.08	11.96 ±0.95
Spleen	26.29 ±1.33	3.59 ±0.64	2.73 ±0.36	1.40 ±0.14	17.60 ±0.73	24.80 ±2.21	4.88 ±0.61	3.10 ±0.29	0.96 ±0.05	14.57 ±1.18
Heart	28.94 ±1.86	6.42 ±0.82	1.45 ±0.10	0.68 ±0.13	18.95 ±1.46	27.46 ±2.34	5.84 ±0.73	1.74 ±0.12	0.92 ±0.15	19.33 ±1.33
Muscle	28.76 ±1.59	7.67 ±1.09	0.80 ±0.07	0.65 ±0.31	18.77 ±1.05	39.31* ±2.61	8.98 ±0.80	1.03 ±0.21	0.88 ±0.09	27.06* ±1.57
Testis	17.74 ±1.85	4.86 ±0.95	0.45 ±0.04	0.51 ±0.06	11.58 ±0.78	18.75 ±1.14	3.72 ±0.68	1.07 ±0.33	1.10 ±0.28	10.88 ±0.93

N.F: Nuclear fraction, M.F: Mitochondrial fraction, Mc.F: Microsomal fraction, C.F: Cytosolic fraction.
Mean value significantly different from that of control value: * $p<0.05$, ** $p<0.01$.

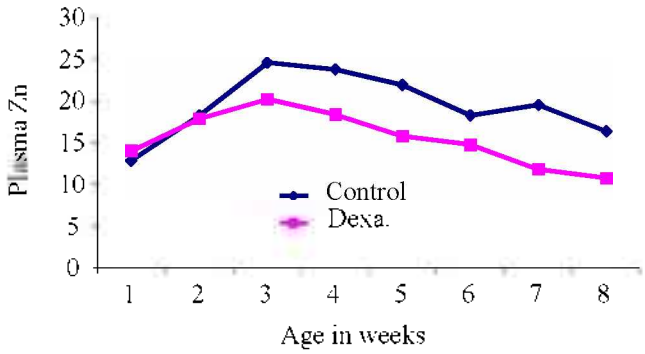

Figure 1. Mean plasma Zn concentration (μmol/L) in neonatal buffalo calves following daily i/m administration of dexamethasone.

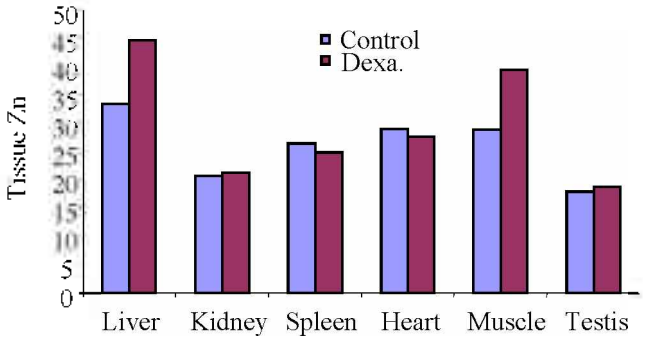

Figure 2. Mean Zn concentration (μg/g wet tissue) in various tissues of neonatal buffalo calves following daily i/m administration of dexamethasone.

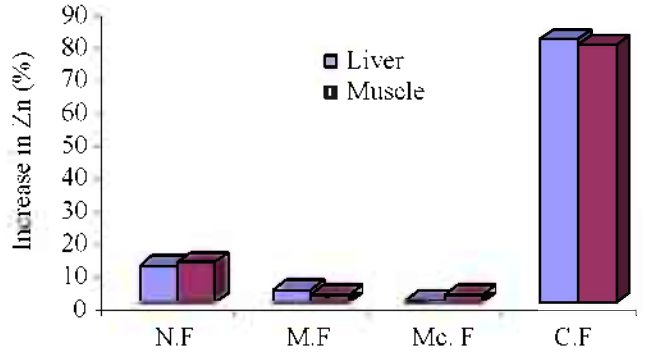

Figure 3. Percentage of the increased Zn in various subcellular fractions.

fractions (table 1) of above mentioned tissues, showed highest concentration in cytosolic fractions followed by nuclear fractions. Mitochondrial and microsomal fractions had comparatively low Zn levels. These results were generally in accordance with those carried out by other workers (Saylor et al., 1980; Laflamme et al., 1985; Jenkins, 1989).

Administration of dexamethasone resulted in a significant ($p<0.05$) increase in Zn levels of liver and muscle. On the basis of Zn concentration in sub cellular fractions, there was significant ($p<0.05$) increase only in the cytosolic fractions of liver and muscle Of the increased zinc, 11.2% and 12.4% was observed in the nuclear fractions; 80.2% and 78.5% in the cytosolic fractions of liver and muscle respectively (figure 3). Mitochondrial and microsomal fractions of these tissues showed only marginal increase. Zinc concentrations of other tissues were not significantly different from the values of control group (figure 2).

These findings can be explained on the basis of role played by liver in zinc metabolism. Once infused into the venous system, zinc is cleared within 3 h (Montgomery et al., 1943; Vikbladh, 1950). It is taken up by liver and eventually appears in pancreas, kidneys and other tissues, suggesting that liver may be central to zinc transfer and distribution (Endre et al., 1990). Zinc uptake by liver from plasma may be among the earliest steps in the body's response to a number of physiological stimuli. Hormones like corticosteroids or estrogen lower plasma zinc by 30-40% and this persists as long as the hormones continue to be administered (Falchuk, 1977; Flynnn et al., 1971; Henkin, 1974). All of the hormone's effects are believed to be associated with an increase in zinc uptake by liver. Further, synthesis or release of leukocyte endogenous mediator (LEM), a Zn-binding ligand, is reported to be increased by stress, causing an increase in liver uptake of zinc, resulting in decreased plasma Zn (Brewer and Prasad, 1977). Therefore, calves stressed by continuous administration of glucocorticoid injections might be expected to show increased Zn uptake by liver.

Most of the increased zinc in liver and muscle appeared in their cytosolic fractions. This could be due to the binding of zinc to metallothionein. A number of physiological agents including hormones like dexamethasone, induce metallothionein synthesis *in vivo* (Vallee and Falchuk, 1993). The excess Zinc, which accumulates in the cytosolic fraction of liver almost exclusively bind to this low molecular weight zinc binding protein called metallothionein & it has been reported in rat (Chen et al., 1974), calf (Kincaid et al., 1976) and goat kids (Tanabe, 1980). Metallothionein has been thought to regulate zinc metabolism.

Results of this study pointed towards alteration in Zn metabolism and homeostasis in neonatal buffalo calves, which were under stress due to continuous administration of glucocorticoid (dexamethasone) injection. Increase in Zn uptake by liver led to reduced plasma Zn levels, indicating a redistribution of zinc within the body. From liver zinc might appear in other tissues like muscles.

REFERENCES

Brewer, G. J. and A. S. Prasad. 1977. Zinc metabolism : Current Aspects in Health and Disease. Alan. R. Liss, Inc., New York.

Carcagno, A. R., F. A. Gullace, I. J. Soler, C. A. Fernandez, A. M. Bernardi and E. G. Capaul. 1994. Zinc in blood plasma and hair concentration and distribution in 150 Aberdeen Angus steers. Veterinary Bulletin 64(12):7983(Abstr.).

Chen, R. W., D. J. Eakin and P. D. Whhanger. 1974. Biological function of metallothionein II. Its role in zinc metabolism in rat. Nutr. Rep. Int. 10:195.

Endre, L., F. W. J. Beck and A. S. Prasad. 1990. The role of zinc in human health. J. Trace Elem. Exp. Med. 3:337-375.

Falchuk, K. H. 1977. Effect of acute disease and ACTH on serum zinc proteins. N. Engl. J. Med. 269:1129-1134.

Flynn, A., W. J. Pories, W. H. Strain, O. A. Hill and R. B. Fratianne. 1971. Rapid serum zinc depletion associated with corticosteroid therapy. The Lancet. 27:1169.

Henkin, R. I. 1974. On the role of adrenocorticosteroids in the control of zinc and copper metabolism in man. In: Trace Elements Metabolism in Animals (Ed. W. G. Hoekstra, J. W. Suttie, H. E. Gonther and W. Mertz. Baltimore). M.D.: University Park, 1974. Vol. 2, p. 674.

Jenkins, K. J. 1989. Effect of copper loading of pre ruminant calves on intra cellular distribution of hepatic copper, zinc, iron and molybdenum. J. Dairy Sci. 72:2346-2350.

Kincaid, R. L., W. J. Miller, R. P. Gentry, D. L. Hampton and M. W. Neathery. 1976. Effect of high dietary Zn upon zinc metabolism and intracellular distribution in cows and calves. J. Dairy Sc. 59 (9):1580-1584.

Laflamme, D. P., W. J. Miller, M. W. Neathery, R. P. Gentry, D. M. Blackmon, K. R. Longer and A. S. Fielding. 1985. The effect of low to normal dietary phosphorus levels on zinc metabolism and tissue distribution in calves. J. Anim. Sci. 61(2):525-531.

Ludmilla, D. 1976. Chemical analysis by atomic absorption spectroscopy. Varian Techtron Pvt. Ltd. Melbourne, Australia.

Miller, W. J., E. S. Wells, R. P. Gentry and M. W. Neathery. 1971. Endogenous Zn excretion and ^{65}Zn metabolism in Holstein Calves fed intermediate to high but non toxic Zn levels in practical diets. J. Nutr. 101:1673-1682.

Montgomery, M. L., G. H. Sheline and I. L. Chaikoff. 1943. Elimination of administered zinc in pancreatic juice and bile of dog as measured by its radioactive isotope (Zn^{95}). J. Exp. Med. 78:151-156.

Porter, H., W. Wiener and M. Barker. 1961. The intra cellular distribution of Copper in immature liver. Biochem. Biophys. Acta. 52:419.

Sawadogo, G., P. Saqui-Sannes and V. Bugat. 1988. Note on the effects of age and sex on plasma concentration of copper, zinc and magnesium in Gobra Zebus. Veterinary Bulletin 58(8): 5049(Abstr.).

Saylar, William, W. and Ronald, M. Leach. 1980. Intracellular distribution of copper and zinc in sheep : effect of age and dietary level of the metals. J. Nutr. 110:448.

Snedecor, G. W. and W. G. Cochran. 1976. Statistical Methods. Iowa. Iowa State University Press, USA.

Tanabe, S. 1980. Effect of zinc injection on zinc binding in cytosols of several tissues of kids. Br. J. Nutr. 44:355.

Vallee, B. L. and K. H. Falchuk. 1993. The biochemical basis of zinc physiology. Physiol. Rev. 73(1):92.

Vikbladh. I. 1950. Studies on zinc in blood. Scand. J. Clin. Lab. Invest. 2:143-148.

Weeks, B. R., J. E. Smith, R. M. DeBower and J. M. Smith. 1989. Decreased serum iron and zinc concentration in cattle receiving intravenous dexamethasone. Vet. Pathol. 26(4):345-346.

Wegner, T. N., D. E. Ray, C. D. Lox and G. H. Stott. 1973. Effect of stress on serum zinc and plasma cortcoids in dairy cattle. J. Dairy Sci. 56(6):748-752.