Afr. J. Med. med. Sci. (1998) 28, 189-192

Effect of dietary zinc deficiency on alkaline phosphatase and nucleic acids in rats

[a] E.O.Okegbile , [b] O. Odunuga and [c] A.Oyewo
[a,b] *Department of Biochemistry, Ogun State University, Ago-Iwoye, Nigeria.*
[c] *Department of Biochemistry, University of Ilorin, Ilorin, Nigeria*

Summary
Weanling male albino rats were randomly alloted to zinc deficient fed (ZnDF) pair-fed (ZnPF) or ad libitum-fed (ZnAL) dietary treatments. The rats were fed diets with either low (5 µg/g) or adequate (100 µg/g) zinc for 28 days. Zinc deficiency significantly reduced growth rate by 60% and was associated with a significantly low feed intake when compared with ZnPF and ZnAL groups. DNA and RNA contents of the liver were used as indication of nitrogen metabolism. DNA content was similar for both ZnPF and ZnAL groups (1.90 and 2.20 mg/g wet weight, respectively), but significantly different from ZnDF (1.42 mg/g wet weight). Liver RNA values of ZnAL, ZnPF and ZnDF groups similarly varied (25.0, 20.2 and 14.8 mg/g wet weight, respectively). Liver, muscle, spleen, femur and serum zinc concentrations were lowest in rats fed ZnDF relative to adequate zinc levels. The levels of the alkaline phosphatase activity was highest in the serum and lowest in the brain (spleen value was greater than that of the liver). Alkaline phosphatase activity was similar in ZnAL and ZnPF groups, but significantly different from ZnDF. In conclusion, the constitutively expressed growth rate, DNA level, RNA level, organ/serum zinc contents and alkaline phosphatase activities were markedly affected by zinc deficiency in rats.

Keywords: *Zinc, alkaline phosphatase, necleic acids, rats.*

Résumé
Les rats male de soueches albino nonvellement sevrè ont Ètè attribuès de maniere arbitraire Zinc (ZnDF), pair-fed (ZnPF) on addition illimitè de Zinc-alluminium (XnAI). Les rats ont ètè nourrient avec des aliments contenant soit une faible concentration (5ug/g) ou une concentration adequate (100ug/g) de Zinc prenatant fours La deficience en Zinc a reduit significativement le taux de croissance des rats de 60&, et a ètè associè `a une perte d'appetie comparè aux animaux nourrient avel le ZnPF et ZnAL. Les contenus d'AND et l'ARN du foie ont ètè utulisès t' d'AND ont ètè similaires dans les groupes d'animaux traitès à la ZnAL et ZnAL (1.90 et 2.20mg/g de poids frais respectivement). Les concentrations de Zinc dans les foies, les muscles, la r`ate, le femur,et le serm ont ètè faibles chez rats traites à la ZnDF. Les taux d'activitès d'alkaline phosphatase ont ètè plus forte dans le serum et plus faible dans le cerveaux (le taux d'activite dans la ràte a ètè plus forte que celle du foie).

L"activitè de l'alkaline phosphatase a ètè similaire dans les groupes traitès au ZnAL et ZnPF mais a ètè significantivement differente comparè aux les animaux traitès avec le ZnDF. En conclusion, le taux de croissance, le taux d'AND, le taux d'ARN, le contenu de Zinc dans le serum et les organes et l'activite de la phosphatase alkaline ont ètè serioueusement affectès par lar deficience en Zinc chez les rats.

Correspondence: E.O. Okegbile, Dept. of Biochemistry, Ogun State University, Ago-Iwoye, Nigeria.

Introduction

Zinc (Zn) is an essential trace element for all species studied to date including humans [1,2]. As one of the most important trace elements, Zn possesses some advantageous characteristics in term of binding strength and rate of ligand exchange that make it a potential source of interesting biological property [3]. In both blood and intracellular fluid, Zn is largely bound to protein. In metalloproteins, Zn is bound with high affinity via three or four cysteine, histidine, glutamate and/or aspartate residues [3].

The metabolism of Zn in mammals is dominated by the lack of highly selective carrier for Zn in the blood and by the existence of metallothioneins, a family of intracellular Zn-binding proteins [2]. In most cell types, a portion of intracellular Zn exists in the form of a Zn metallothionein complex and has been proposed to have a role in the control of whole body Zn metabolism via its action in the liver and intestine.

Zn as a cofactor for a variety of Zn metalloenzymes has also been established as a structural component of DNA-binding proteins that contain Zn fingers, clusters and twists [4]. The pathology resulting from Zn deficiency has been reported to be caused principally by a loss of Zn from the cell plasma membrane [5]. Cellular structures that are in physical contact with extracellular Zn pool will be the first to lose Zn in dietary Zn defiency. The loss of Zn from specific proteins in the cell plasma membranes leads to altered membrane structure and function. The biochemical abnormalities of cells from Zn-deficient animals are a cellular response to the decreased integrity of the plasma membrane. Substantial depletion of the intracellular concentration of functional Zn in mammalian cells would rapidly lead to cell death. The objective of this study was to investigate the effect of dietary Zn deficiency on alkaline phosphatase and nucleic acids in rats.

Materials and methods

Animals

Wealing rats were housed in suspended stainless-steel wire mesh cages. Animals were maintained at 27 ^{0}C with a 12-hr light:dark cycle. Deionized water was provided at all times. The body weight and food intake were measured daily.

Design

Eighteen rats initially weighing 35 ± 0.8 g were allotted to three groups. One group consumed a low Zn (5 µg Zn/g) diet (ZnDF), the diet supplemented with 100 µg Zn/g diet *ad libitum* (ZnAL) and the supplemeted diet, pair-fed (ZnPF). The pair-fed rats were allotted daily the amount of food consumed the previous day by their pair mates. The composition of the diet is shown in Table 1. This diet by analysis contained less than 1 µg Zn/g diet. The control diet was supplemented with 100 µg Zn/g diet as $ZnCO_3$.

Table 1: Composition of diets[1]

Ingredient	Concentration g/kg diet
Egg white solids	200.0
Dextrose monohydrate	634.2
Corn oil	100.0
Cellulose	30.0
Salt mixture[2]	25.7
Vitamin mixture[3]	10.0[4]
Biotin	0.00[4]
Ethoxyquin (antioxidant)	0.02

[1]Shown is the zinc-deficient diet containing $ZnCO_3$, 0.0089 g/kg. In the zinc adequate diet, zinc carbonate ($ZnCO_3$, 0.1786 g/kg) replaced an equal mass of cellulose.

[2]Salt profile (g/kg diet): $CaHPO_4$, 19.7670; $MgSO_4$, 2 452; KCl, 2.2882; NaCl, 0.7781; $FeSO_4$; $7H_2O$, 0.2; $MnSO_4.H_2O$, 0.1662; $CuSO_4$, 0.0004; CrK $(SO_4)_2$ $12H_2O$, 0.02.

[3]Vitamin profile (mg/kg diet): p-aminobenzoic acid, 110.1, ascorbic acid, coated (97.5%), 1016.6; biotin, 0.44; Vitamin B-12 (0.1% trituration,), 29.7; calcium pantothenate, 66.1; choline dihydrogen citrate, 3496.9; folic acid, 1.98; inositol, 110.1; menadione, 49.5; niacin, 991; pyridoxine HCl, 22.0 riboflavin, 22.0; thiamin HCl, 22.0; dry retinyl palmitate, 39.65; dry cholecalciferol, 4 4; dry dl-x0tocopheryl acetate, 242.3.

[4]Corn starch (46.66%) serves as a diluent for the vitamin mixture.

Tissue collection and preparation

On day 29, rats were anaesthetized with chloroform and blood was collected from the abdominal aorta. Serum was obtained by centrifugation and stored at 20 °C until analysis.

The liver, spleen, brain, tibia and muscle were removed. Pieces of liver, spleen and brain were immediately homogenized with phosphate-buffered saline (PBS) pH 7.3. Homogenate was obtained by centrifugation and stored at –20 °C for alkaline phosphatase assay. Tibias were stored at –20 °C.

Alkaline phosphatase assay

The alkaline phosphatase activity of serum and tissue homogenates were determined a the Chemical Pathology Department, University College Hospital (UCH), Ibadan using Boehringer Mannheim GMBH automated analyzer. BM/Hitachi system 704.

Zinc analysis

Tissues and tibias were digested in tetraoxonitrate (V) acid [6]. The digests were diluted to 10 ml with deionized distilled water. Serum for Zn analysis was diluted 1:4 with deionized water and analyzed directly. Zn was determined in triplicate by flame atomic absorption spectrophotometry.

Estimation of DNA and RNA

Tissues were homogenized in trichloroacetic acid. DNA contents were determined by the method of Burton [7], while RNA contents were determined as described by Santon and Agranoff [8].

Statistics

Means and standard errors were determined. Significant differences between the means ($P < 0.05$) were determined using Duncan's multiple range test.

Results

The nutritional status of rats fed the experimental diet was evaluated by feed efficiency and weight gain during the experimental period and the terminal tissue and serum zinc concentrations. The Zn-deficient rats displayed anorexia and in action that are common with Zn deficiency. Food intake was reduced by 25% when compared with the *ad libitum-fed* rats (Table 2).

Table 2: Final body weight, growth, food intake, feed efficiency and liver weight in zinc-deficient, pair-fed and ad libitum-fed rats[1]

	ZnAL	ZnPF	ZnDF
Final body weight (g)	126.6[b] ± 5.9	76.5[a] ± 6.5	70.3[a] ±3.9
Growth rate (g/d)	4.3[b] ± 0.2	2.0[a] ± 0.2	1.9[a] ± 0.93
Food intake (g/d)	8.3[b] ± 0.4	6.60[a] ± 0.4	6.60[a] ± 0.3
Feed efficiency[2]	0.49 ± 0.04	0.14[a] ± 0.03	0.11[a] ± 0.05
Liver weight (g)	5.5[b] ± 0.6	2.6[a] ± 0.2	2.5[a] ± 0.4

[1]*Values are means, n = 6 ($P < 0.05$)*
[2]*Body weight gain g food intake.*

Consequently, average daily gain in weight of Zn deficient and pair-fed rats were 44% and 47% that of ZnAL control group, respectively. There was no significant difference in the average daily weight gained in ZnDF and ZnPF rats. The feed efficiency of the Zn-deficient rats and their pair-fed counterparts were significnatly lower than that of the ZnAL control-fed group, indicating explicit Zn deficiency. The liver weight of the Zn-deficient rats and their pair-fed counterpart were 45% and 47%, respectively, of those of the ZnAL-control-fed group. There were no significant differences in the final body weight, growth rate, food intake, feed efficiency and liver weight between ZnDF and ZnPF rats (Table 2).

Seron Zn cocentration of ZnDF and ZnPF rats were about 49% and 88% of that of the ad libitum-fed control group (Table 3). However, the serum Zn level of ZnDF was found to be about 56% of the value of ZnPF. The concentrations of Zn in the muscle of ZnDF and ZnPF rats were similar but significantly different from those of the ZnAL group.

Table 3: Comparison of liver, muscle, spleen, tibia and serum zinc concentration of zinc-deficient, pair fed and ad libitum-fed rats[1]

	ZnAL	ZnPF	ZnDF
Liver[2]	0.43^b	0.35^b	0.20^a
	± 0.01	± 0.02	± 0.04
Muscle[2]	0.24^b	0.19^a	0.18^a
	± 0.06	± 0.04	± 0.02
Spleen[2]	0.44^c	0.34^b	0.18^a
	± 0.02	± 0.04	± 0.01
Tibia[2]	0.37^b	0.34^b	0.29^a
	± 0.03	± 0.10	± 0.03
Serum[3]	13.3^c	11.7^b	6.5^a
	± 0.9	± 0.8	± 1.1

[1]Values are means, n = 56 ($P < 0.05$)
[2] mg/g tissue
[3] µmol/L

Dietary Zn deficiency was observed to lower the activity of alkaline phosphatase (Fig. 1). The activities of alkaline phosphatase in the liver, brain, spleen and serum of ZnDF rats were significantly different from those of both the ZnPF and ZnAL groups of rats. There were however no significant difference between ZnPF and ZnAL groups. Of the tissues investigated, the brain was the most affected as there was a 64% reduction compared to 36% and 12% observed in the liver and spleen, respectively. In the serum, a 54% reduction was observed.

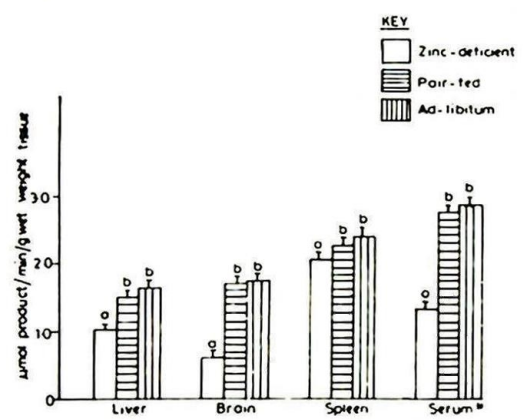

Fig. 1 Comparison of liver, brain, spleen and serum alkaline phosphatase among the zinc-deficient, pair-fed and ad-libitum treatment. Values are means ± S.E.M. Differences $P < 0.05$ were considered significant and are denoted by different letters
* One unit of activity = µMol/min/m

Hepatic RNA and DNA levels were compared among the dietary treatments (Fig. 2). Both the DNA and RNA levels were significantly different at $P < 0.05$ in Zn-deficient fed rats compared to both the ZnPF and ZnAL fed groups. The DNA and RNA values in ZnPF group were not significantly different from those of the ZnAL fed group. However, the RNA/DNA ratios in both ZnDF and ZnPF groups of rats were significantly different from those of the ZnAL control group. The ratio was 90% of the ZnAL in ZnDF and 89% in ZnPF group of rats. The ratio was however not significantly different between ZnDF and ZnPF groups.

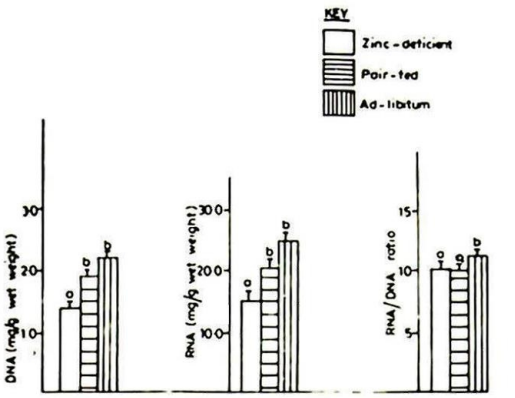

Fig. 2 Comparison of RNA, DNA and RNA/DNA ratio levels among the Zinc-deficient, pair-fed and ad-libitum-fed treatment. Values are means ± S.E.M. Differences of $P < 0.05$ were considered significant and are denoted by different letters

Discussion

Growth retardation is a predominant symptom of Zn deficiency. Within a few days of consuming the Zn-deficient diet, rats became anorectic and growth rate declined. The reduction in growth was caused by a decrease in food intake and lower feed efficiency (Table 2). We found that lower serum, tibia and some tissue zinc concentrations were also associated with Zn deficiency though reductions were also observed in the ZnPF group (Table 3). This may be due to the mobilization of Zn from exchangeable pool to support high-priority tissues such as muscle and skin [9]. Hence, a decrease in a family of intra-cellular Zn-binding proteins [10] metallothioneins and low molecular weight substances [11]. Previously, metallothioneins were said to have a role in the control of whole body Zn metabolism via their action in the liver and intestine [10,12]. Furthermore tissue metallothionein concentrations have been reported to be directly proportional to Zn status [9].

In the present study, the liver, brain, spleen and serum alkaline phosphatase showed markedly different levels of reduction due to Zn deficiency. The levels of alkaline phosphatase activity in ZnDF group of rats were about 64,36,88 and 46 percent of those of the ZnAL-fed group of rats, respectively. However, the level of the enzyme activity in the ZnPF group was not significantly lower compared to ZnAl groups. The variable effect of dietary Zn deficiency on the activity of alkaline phosphatase may possibly be due to different turnover rates of the enzyme in different tissues or a difference in the rate of depletion of the pool of Zn with each tissue or substitution for Zn without loss of enzyme activity [13]. Alkaline phosphatase, a Zn metalloenzyme by definition, has tighly bound Zn, and one would expect it would be the last molecule to loose Zn during dietary Zn deprivation [3].

Our study has revealed that feeding rats a low Zn diet for four weeks markedly reduces Zn content of some tissues, serum and alkaline phosphatase activity. A positive correlation may exist between alkaline phosphatase activity and tissue Zn concentrations and Zn levels. In a previous report [14], plasma Zn concentration, coupled with measurement of alkaline phosphatase activity, has been a suggested useful index of Zn deficiency.

The levels of DNA and RNA in the liver of both ZnDF groups were about 62 and 60 per cent of ZnAL,

respectively. It has been reported that many metals interact specifically in both RNA and DNA, suggesting that these metals play a role in the maintenance of the configuration of RNA. The maintenance perhaps, is by linking the purine and pyrimidine base or both through covalent bonds possibly involving nitrogen atoms or π (pic) electrons of the bases [15]. In this study, it is pertinent to report that Zn (as a micronutrient) either singly or in combination with other metals (cations) may affect the configuation of specific interaction in both RNA and DNA, thus accounting for a decrease in the nucleic acid content in dietary Zn deficient rats.

Furthermore, Zn has been reported to be a cofactor of a variety of Zn metalloenzymes involved with nucleic acid metabolism and as a structural component of DNA-binding proteins that contain Zn fingers, cluster and twists [3,16]. Thus, the observed variation in the hepatic RNA, DNA and RNA ratio may be attributable to dietary Zn deprivation and disruption in normal patterns of protein synthesis [17]. In the present study, Zn probably affects the expression of genes involved in nucleic acids intracellular signalling pathway [19]. The alteration in gene expression in Zn-deficient rats may be due to a lack of Zn in Zn metalloproteins involved with the transcription and/or translation.

Acknowledgements

We are grateful to Mrs. V.O. Ogunbanjo for technical assistance. The secretarial service of Mrs. I.O. Ogunboyejo is also appreciated.

References

1. Underwood EJ. Trace elements in human and animal nutrition. 1962; p. 173. Academic Press, New York, NY USA.
2. Walsh CT, Sandstead HH, Prasad AS, Newbeme PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 1994; 102 (suppl. 2): 5-46.
3. Bettger WJ, O'Dell BL. Physiological roles of Zinc in the plasma membrane of mammalian cels. J Nutr Biochem 1993; 4: 194-207.
4. Shay NF, Cousins RJ. Cloning of rat intestinal mRNAs affected by Zinc deficiency. J Nutri 1993; 123: 35-41.
5. Fortes C. Aging, zinc and cell-mediated immune response. Aging Clin Exp Res 1995; 7: 75-93.
6. Clegg MS, Keen CL, Lonnerdahl B, Hurley L. Influence of ashing techniques on the analysis of trace elements in animal tissues. I. Wet ashing. Biol Trace Elem Res 1981; 3: 107-115.
7. Burton K. A study of the conditions and mechanism of the diphenlamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956; 62: 315-323.
8. Santen RJ, Agranoff BW. Studies of the estimation of deoxyribonucleic acid and ribonucleic acid in rat brain. Biochemica et Biophysica Acta. 1963; 72: 251-262.
9. King JC. Assessment of zinc status. J Nutr 1990; 120: 1474-1479.
10. Cousins RJ. Absorption , transport and hepatic metabolism of copper and zinc: Special - reference to metallothionein and ceruloplasm Physiol Rev 1985; 63: 238-309.
11. Faure H, Favier A, Tripier M, Arnaud J. Determination of the major zinc fractions in human serum by ultra-filtration. Biol Trace Elem Res 1990; 24: 25-37.
12. Hempe JM, Carlson JM, Cousins RJ. Intestinal metallothionein gene expression and zinc absorption in rats are zinc-responsive but refractory to dexamethasone and interleukin 1. J Nutr 1991; 121: 1389-1396.
13. Roth HP, Kirchgessner M. Zn metalloenzyme activites. Changes and biochemical aspects in Zn deficiency. World Rev Nutr Diet 1980; 34: 144-160.
14. Gibson L. Zinc nutrition in developing countries. Nutr Res Rev 1994; 7: 1510173.
15. Cousins RJ. Regulatory aspects of zinc metabolism in liver and intestine. Nutr Rev 1979; 37-107.
16. Valle BL, Palchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993; 73: 79-118.
17. Ilabert ER, Ruiz E, Osorio C, Ortega E. Effect of dietary zinc deficiency on reproductive function in male rats: Biochemical and morphometric parameters. J Nutr Biochem 1996; 7: 403-407.
18. Mcnall AD, Etherton TD, Fosmire GJ. The impaired growth induced by zinc deficiency in rats associated with decreased expression of the lepatic insulin-like growth factor 1 and growth hormone receptor genes. J Nutr 1995; 125: 874-879.