Zinc-deficiency and activities of urea cycle-related enzymes in rats[1]

M. Rahmatullah, Louise Y.Y. Fong, J.S.K. Lee and T.R.C. Boyde

Department of Biochemistry, University of Hong Kong, Hong Kong (Hong Kong), 12 February 1980

Summary. In contrast to previous reports, an increase in glutamate dehydrogenase activity and no change in arginase activity were observed in rats fed a zinc-deficient diet for 15 weeks. The discrepancies could be due to a difference in degree and duration of zinc-deficiency.

Several investigations have indicated that defects in urea cycle enzyme systems might be present in rats fed a zinc-deficient diet. Excessive amounts of nitrogen, urea and uric acid were found in urine of rats fed a zinc-deficient diet for 2–3 weeks accompanied by an increase in arginase activity[2]. On the other hand, no change in arginase or glutamate dehydrogenase activity was observed in pigs after 6 weeks of a zinc-deficient diet[3]. Rabbani et al.[4] found that blood urea nitrogen in zinc-deficient rat declined sharply and significantly during the 4th week of the dietary regimen. It appeared that duration of zinc-deficiency might affect activities of these enzymes. This led us to measure the levels of ornithine carbamoyltransferase (OCT), arginase, glutamate dehydrogenase (GDH) and aspartate aminotransferase (AAT) in rats fed a deficient diet for a prolonged period.

Materials and methods. Animals. Male, weanling rats (50–55 g) of Sprague-Dawley strain obtained from the Animal Laboratory Unit, University of Hong Kong were divided randomly into 2 groups. They were housed in plastic cages[5]. Experimental animals were fed ad libitum a zinc-deficient diet[5] using EDTA-washed soybean protein as the protein source. The zinc-deficient diet contained 5–6 ppm of zinc as determined by atomic absorption spectroscopy. Controls were pair-fed the same diet except that supplementary zinc sulphate was given to provide a dietary zinc level of 100 ppm. Deionized water was supplied to both groups. These animals were maintained for 15 weeks and were then killed by cervical dislocation. Livers, kidneys and small intestines (a 10-cm segment distal to the stomach) were removed quickly. Activities of enzymes were determined in tissues where they have maximum activities.

Determination of enzyme activities. OCT; Triethanolamine buffer (0.27 M, pH 7.7) containing 2.5 mM ornithine and 5 mM carbamoylphosphate was used for assay of OCT activity[6]. Arginase was assayed in arginine-glycine buffer (pH 9.5) at 37 °C following activation of tissue homogenates for 10 min at 52 °C[7]. Assays for AAT[8] and GDH[9] were followed by measuring decrease of absorbance at 340 nm in a LKB 2086 Reaction Rate Analyzer (LKB Instruments Ltd, Bromma, Sweden).

Results and discussion. After being fed a deficient diet for 2 weeks, the rats began to develop deficiency symptoms characterized by growth retardation, hair loss, dermal lesions and fissures at the mouth corners. Pair-fed controls showed none of these signs. The 5–6 ppm of zinc in the deficient diet permitted a chronic rather than acute (probably early) lethal deficiency. Before the animals were killed, serum zinc levels, determined by atomic absorption spectroscopy, were 0.27 ± 0.07 and 0.95 ± 0.06 µg/ml respectively for zinc-deficient and control rats. This difference was significant at the $p < 0.001$ level.

Results presented in the table showed that zinc-deficiency had no effect on tissue weight and tissue protein content. The level of intestinal OCT in zinc-deficient rats is not significantly lower than that in the controls ($p \leqslant 0.05$). The reason might be that the contribution of intestinal mucosa to ammonia utilization in the animal as a whole is not significant[10,11]. On the other hand, zinc-deficient rats showed significantly lower liver OCT and higher liver GDH activities as compared to those in controls ($p < 0.01$ in both cases). These data lent support to the observation of Rabbani et al.[4] that ammonia utilization was defective in zinc-deficient rats and resulted in its elevation in the plasma. These authors also noted a diminished hepatic OCT activity but did not measure GDH. Though bovine GDH has earlier been considered a zinc metalloenzyme[12], Colman et al.[13] showed that zinc was not an essential constituent of the enzyme but functioned as an inhibitory allosteric modifier. Therefore, it might be possible that under prolonged and severe deficiency there would be less free zinc available in the tissue for interaction with the enzyme, and as a result, GDH activity would be increased. The fact that other investigators observed no change in hepatic GDH levels in their deficient animals[2,14] might be due to a shorter and less severe deficiency. The same argument could apply to our observation of no change in hepatic arginase activity, while Hsu et al.[2] reported an

The effect of zinc-deficiency on activities of OCT, GDH, arginase and AAT

Enzyme	Tissue	Unit*/g fresh wt		Unit*/mg protein	
		Pair-fed control	Zn-deficient	Pair-fed control	Zn-deficient
OCT	Liver	210.13 ± 16.80	124.48 ± 25.82**	1.01 ± 0.03	0.66 ± 0.11**
	Intestine	9.08 ± 2.52	5.97 ± 2.73	0.13 ± 0.01	0.08 ± 0.03
GDH	Liver	139.41 ± 22.60	231.50 ± 29.61**	0.71 ± 0.11	1.17 ± 0.17**
	Kidney	26.68 ± 10.12	28.59 ± 6.91	0.24 ± 0.08	0.25 ± 0.06
arginase	Liver	572.93 ± 45.75	528.03 ± 118.42	3.00 ± 0.25	2.73 ± 0.37
	Intestine	39.53 ± 13.73	33.22 ± 14.55	0.60 ± 0.10	0.49 ± 0.20
AAT	Liver	215.34 ± 35.79	280.44 ± 37.80	1.03 ± 0.11	1.47 ± 0.26
	Kidney	94.53 ± 8.82	80.00 ± 11.06	0.86 ± 0.11	0.71 ± 0.08

* Unit is expressed as μmole product liberated per min; ** significantly different from value for pair-fed control ($p < 0.01$). The data is presented as the mean ± SD of at least 10 rats.

increased activity. These authors killed their rats after a 2–3 week dietary regimen and a recent report[4] indicated that blood urea nitrogen started to decline sharply and significantly at the 4th week. Obviously, further studies are needed to ascertain the role of zinc in the urea cycle.

1 We wish to thank Professor P.M. Newberne for supplying the plastic cages and ingredients for the zinc-deficient diet.
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