INCREASED PURINE NUCLEOTIDE CYCLE ACTIVITY ASSOCIATED WITH DIETARY ZINC DEFICIENCY

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SUMMARY: Rat muscle 5'-AMP aminohydrolase (EC 3.5.4.6), adenylosuccinate synthetase (EC 6.3.4.4), and adenylosuccinate lyase (EC 4.3.2.2) activities were elevated 50-60% in zinc-deficient weanling rats when compared with restricted-fed zinc supplemental control rats. In addition, the activities of these enzymes were increased by 50-100% when zinc-deficient rats were compared with ad libitum-fed controls. There was no significant difference in total muscle protein or total muscle zinc among the three groups of animals. This increased activity of the purine nucleotide cycle may be responsible for the recently observed increase in blood ammonia in zinc-deficient rats when compared to controls.

INTRODUCTION

Zinc deficiency produces a variety of enzyme responses in various tissues (1-4). There is recent evidence that an increase in protein catabolism is associated with zinc deficiency (5, 6). In view of the proposal that AMP deaminase may be involved in muscle amino acid catabolism (7) and the fact that the enzyme from several sources has been shown to be a zinc metalloenzyme (8, 9) we have investigated the effects of zinc deficiency on rat muscle AMP deaminase activity. In addition, recent reports of reduced plasma aspartic acid levels (10) as well as elevated plasma ammonia levels (11) in zinc-deficient rats as compared with zinc-supplemented rats were suggestive of increased activity of the other enzymes of the purine nucleotide cycle (12, 13), adenylosuccinate synthetase and adenylosuccinase. We have therefore measured the activities of these enzymes in muscle homogenates from zinc-deficient, pair-fed zinc-supplemented, and ad libitum-fed zinc-supplemented rats.

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MATERIALS AND METHODS

Male weanling rats of the Sprague-Dawley strain were obtained from a local breeding farm at 21 days of age and immediately placed on experiment. Rats were kept in individual stainless steel cages with free access to deionized distilled water. The zinc-deficient diet has been previously described (3). This diet contained, by analysis, 0.6 ppm zinc. Positive control diets contained added zinc carbonate to provide an additional 50 ppm of zinc. In the first experiment eighteen rats were divided into three equal groups. In the second experiment thirty rats were divided into three equal groups. For each experiment group 1 received the low zinc diet; group 2 the zinc-supplemented diet but with the intake restricted to the average amount consumed daily by group 1; group 3 received the zinc-supplemented diet in unrestricted amounts. After the three week experimental feeding period rats were killed by decapitation and upper hind leg muscle was dissected out and frozen at -20°C.

Frozen muscle samples were thawed and individually homogenized in exactly 3.3 volumes of ice cold extraction buffer containing 0.18 M KCl, 0.054 M KH_2PO_4, 0.035 M K_2HPO_4, pH 6.5. Extracts were stirred occasionally for one hour at room temperature and were then centrifuged at 14,000 x g for 20 minutes. The supernatant was filtered through Pyrex wool and all assays were performed on this fraction.

AMP, IMP, GTP, aspartic acid, HEPES and MES were all purchased from Sigma Chemical Co. Adenylosuccinic acid was from Calbiochem. Enzyme assays were performed as follows: The conversion of AMP to IMP was followed spectrophotometrically at 265 nm. Adenylosuccinate synthetase and adenylosuccinase were assayed at 280 nm. AMP deaminase was assayed at 30°C with either 0.05 mM AMP (experiment 1) or 0.10 mM AMP (experiment 2), 0.1 M KCl, 0.05 M MES-Tris, pH 6.5. Adenylosuccinase was also assayed at 30°C with 0.05 mM adenylosuccinate, 10mM potassium phosphate, pH 7.0. Adenylosuccinate synthetase was assayed at 37°C in an assay mixture containing 5 mM aspartic acid, 0.15 mM IMP, 0.06 mM GTP, 1 mM $MgCl_2$, 0.05 M HEPES, pH 7.5. Basal rates of reaction prior to the addition of an aliquot of extract were subtracted from post-addition rates to yield synthetase activity. Protein determinations were by the method of Lowry et al (14) using bovine serum albumin as a standard.

Atomic absorption analysis was performed using a Perkin-Elmer 303 Atomic Absorption Spectrophotometer and Harleco standardized zinc solutions. The muscle samples for analysis were ashed in a mixture of nitric acid and perchloric acid. Data were examined by analysis of variance, with statistical significance of treatment differences being determined by the multiple range test (15).

RESULTS

Table I shows the effect of zinc deficiency on total body weight and diet consumption. The growth of the zinc-deficient rats is severly depressed. Growth of the restricted-fed control group is less than that of the ad libitum-fed controls but significantly greater than the zinc-deficient group.

TABLE I: Effect of Zinc Deficiency on Growth and Diet Consumption of Rats.

Group	Initial Weight (g)	Final Weight (g)	Average Daily Diet Consumed (g)
Experiment 1*			
1. Zinc-deficient	54.7 ± 1.8	68.5 ± 3.7	4.9 ± 0.3
2. Restricted-fed control	54.5 ± 1.9	92.8 ± 5.2[a]	4.9 ± 0.1
3. Ad libitum-fed control	54.7 ± 1.9	190.8 ± 12.1[b]	12.6 ± 0.6[b]
Experiment 2**			
1. Zinc-deficient	56.2 ± 1.0	72.2 ± 1.6	5.3 ± 0.1
2. Restricted-fed control	55.8 ± 1.0	91.1 ± 0.8[a]	5.3 ± 0.1
3. Ad libitum-fed control	56.2 ± 0.9	200.5 ± 2.5[b]	13.2 ± 0.2[b]

* Each group value is the average for six rats with the standard deviation shown.

**Each group value is the average for ten rats with the standard deviation shown.

[a] Significantly greater than least value ($P < 0.01$).

[b] Significantly greater than least two values ($P < 0.01$).

Table II summarizes the results of experiment 1. There is no significant difference in whole muscle zinc levels or homogenate protein concentration among the groups. However, extract AMP deaminase activity is elevated by 50% in upper hind leg muscle of zinc-deficient rats when compared with restricted-fed control animals. This increase cannot be attributed to changes in muscle protein content, but must reflect an increase in the total units of activity of this enzyme in the zinc-deficient rat. Units of activity are increased by 30% when zinc-deficient animals are compared with ad libitum-fed controls.

TABLE II: Experiment 1; Effect of Zinc Deficiency on Whole Muscle Zinc Content and 5'-AMP Aminohydrolase Activity.

Group	Muscle Zinc Content (ppm)	Extract Protein Content (mg/ml)	5'-AMP deaminase Specific Activity[a]
1. Zinc-deficient	8.38 ± 0.93[b]	8.60 ± 2.1	.033 ± .008[c]
2. Restricted-fed control	8.14 ± 0.77	8.70 ± 0.9	.021 ± .003
3. Ad libitum-fed control	9.05 ± 1.13	11.40 ± 1.3	.019 ± .003

[a]Expressed as μmoles AMP deaminated/min/mg protein.

[b]Mean values plus standard deviation for five individuals in group 1; values are for six individuals in groups 2 and 3.

[c]Significantly greater than least two values ($P < 0.01$).

The results of experiment 2 are summarized in Table III. Again, there was no significant difference among homogenate protein concentrations (not shown). It is clear that the activities of all three muscle enzymes constituting the purine nucleotide cycle are significantly elevated when zinc-deficient rats are compared with either restricted-fed or ad libitum-fed zinc-supplemented control animals.

DISCUSSION

The purine nucleotide cycle, as described by Lowenstein (12), results in the net production of ammonia from aspartic acid as illustrated in Figure 1. The observation that the activities of enzymes catalyzing the cycle are elevated in zinc-deficient rats is consistent with the reported increase in plasma ammonia and decrease in plasma aspartate in zinc-deficient rats as compared with controls (10, 11). The reasons for elevated purine nucleotide cycle activity are not

TABLE III: Experiment 2; Effect of Zinc Deficiency on Activities of Enzymes of Purine Nucleotide Cycle

Group	5'-AMP deaminase Specific Activity[a]	Adenylosuccinate Synthetase Specific Activity[b]	Adenylosuccinase Specific Activity[b]
1. Zinc-deficient	.159 ± .023[c]	.483 ± .045[c]	1.083 ± .265[c]
2. Restricted-fed control	.118 ± .015	.304 ± .050	.645 ± .149
3. Ad libitum-fed control	.107 ± .105	.237 ± .054	.618 ± .101

[a]Expressed as μmoles/min/mg protein

[b]Expressed as μmoles/hr/mg protein

[c]Significantly greater than least two values ($P < 0.01$)

clear. Total muscle zinc and total muscle protein are apparently unaffected by zinc-deficiency. Therefore, muscle metabolic responses to zinc deficiency are probably not a result of increased protein catabolism without subsequent reutilization of resulting liberated amino acids. The proposed function of the purine nucleotide cycle in terms of amino acid catabolism (7, 16) does not seem to be the primary reason for increased cycle activity. Despite the evidence for increased protein catabolism in liver of zinc-deficient rats (6) the activity of glutamate dehydrogenase, the major amino acid deaminating enzyme in liver, is unchanged when such rats are compared to controls (4).

Recently evidence has been accumulating for the functioning of the purine nucleotide cycle as a regulator of the energy charge ratio (17, 18). That is, the AMP deaminase reaction may serve to stimulate ATP production by the myokinase reaction in response to sudden drops in the energy charge ratio. Additionally, the effects of components of the purine nucleotide cycle on glycolysis and, specifically, phosphofructokinase activity need be considered. Ammonium ions are known to be effective activators of phosphofructokinase (19) and oscillations

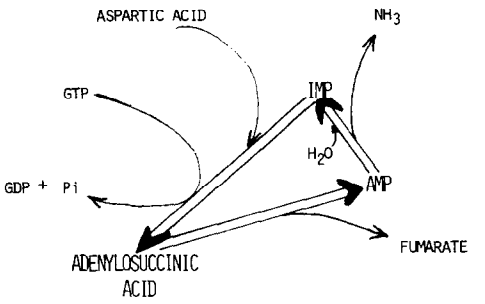

FIGURE 1. THE PURINE NUCLEOTIDE CYCLE INDICATING THE RELATIONSHIP BETWEEN ASPARTIC ACID UTILIZATION AND AMMONIA PRODUCTION

in the purine nucleotide cycle resulting in fluctuations of levels of AMP may have potent effects on phosphofructokinase activity (20). A high correlation between AMP deaminase activity and phosphofructokinase activity among different muscle fiber types adds support to the hypothesis that AMP deaminase activity is a regulator of glycolytic flux (21).

The manner in which zinc deficiency is responsible for alterations in the discussed enzyme activities remains unsolved.

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