Effect of dietary Zn deficiency on 2,3-diphosphoglycerate and adenosine triphosphate concentrations in the rat erythrocyte

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The effect of dietary Zn deficiency on blood concentrations of 2,3-diphosphoglycerate (2,3 DPG) and adenosine triphosphate (ATP) concentration were determined. Weanling male rats in the zinc deficient group were fed an egg white-based diet containing < 1 mg/kg Zn for 3 weeks; control rats were either pair-fed or fed ad libitum the diet supplemented with 100 mg/kg Zn. Dietary Zn deficiency caused a significant elevation of the hematocrit compared to pair-fed and ad libitum-fed controls but did not alter the mean corpuscular hemoglobin concentration. Per unit of whole blood, the feed restriction associated with dietary Zn deficiency resulted in a significant increase in 2,3 DPG and a significant decrease in ATP; per unit of packed red cells, both 2,3 DPG and ATP were significantly decreased.

Keywords: Zn deficiency; erythrocyte; 2,3 DPG; ATP

Introduction

Dietary Zn deficiency in rats is associated with several signs of altered erythrocyte membrane function. Erythrocytes from Zn deficient rats have increased osmotic fragility,[1-4] increased hemolytic sensitivity to sodium dodecyl sulfate, sodium dodecyl n-sarcosine and melittin, and decreased sensitivity to the hemolytic activity of dimethlysulfoxide.[2] The voluntary feed restriction associated with zinc deficiency in rats leads to a significant increase in erythrocyte filterability,[5] and low dietary Zn exacerbates the decreased filterability seen in erythrocytes from vitamin E-deficient rats.[6] Dietary Zn deficiency does not alter peroxidative fragility of erythrocytes,[7] but low dietary Zn does increase the peroxidative fragility of erythrocytes from rats that are also deficient in vitamin E.[6,7] The biochemical basis for these altered membrane properties is unknown.

The effect of dietary Zn deficiency on the structure and function of the erythrocyte may be mediated by changes in the concentration of low molecular weight effectors like divalent cations,[8,9] amino acids,[10] polyamines,[11,12] and polyphosphates.[11] Recently, dietary Zn deficiency has been associated with altered levels of cell polyphosphates. Meftah and Prasad[13] have reported elevated levels of ADP, GTP, and dGTP and lowered ATP/ADP ratios in lymphocytes from human subjects with Zn deficiency. In addition, dietary zinc deficiency in rats leads to elevated dinucleotide 5,5,-diadenosine tetraphosphate concentrations in spleen, an effect apparently associated with the voluntary feed restriction that accompanies the deficiency.[14] Our experiments were designed to determine the effects of dietary zinc deficiency on the concentrations of the two major polyphosphates, 2,3 DPG and ATP, in rat erythrocytes.

Methods and materials

Animals and diet

Male weanling Wistar rats (52–64 g) were obtained from Charles River Laboratories (Montreal, Quebec, Canada). The animals were housed individually in stainless steel cages at 22° C with a photoperiod from 0700–1900 hr. The rats were given free access to doubly deionized water (< 0.01 mg/kg Zn). The composition of the basal diet has been described previously.[5]

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Table 1 Effect of zinc deficiency on rat performance and hematological parameters

	−Zn	+ZnPF	+ZnAL
Weight gain (g/3 weeks)*	6 ± 1[a]	29 ± 3[b]	122 ± 3[c]
Plasma zinc (μg/mL)	0.39 ± 0.03[a]	1.13 ± 0.04[b]	1.33 ± 0.03[c]
Hemoglobin (g/L)	156 ± 3[a]	149 ± 2[a]	131 ± 3[b]
Hematocrit (%)	47.6 ± 0.4[a]	44.7 ± 0.4[b]	39.4 ± 0.3[c]
MCHC (g/L)	328 ± 5[a]	334 ± 4[a]	333 ± 4[a]

Note: Mean ± SEM, $n = 8$, except weight gain. Values of a particular row having different superscript letters are significantly different ($P < 0.05$) by Tukey's test.
* Mean ± SEM, $n = 16$. Mean includes the body weights of those rats raised for plasma Zn determination.

Experimental design

Rats were allocated randomly into zinc deficient (−Zn), pair-fed (+ZnPF), and ad libitum-fed (+ZnAL) treatments. The −Zn rats were fed a basal diet containing < 1.0 mg/kg Zn ad libitum. The basal diet supplemented with 100 mg/kg Zn was fed to each +ZnPF rat, in an amount equal to that consumed by its corresponding −Zn partner during the previous day. The +ZnAL group was fed the zinc-adequate diet (100 mg/kg Zn) ad libitum. The rats were fed their respective diets for 21 days. Rats were raised on three separate occasions; on the first two occasions, the rats were split into two groups, to allow prompt tissue processing. Each group had equal representation from the three diets. For the first group on each occasion, on day 18 and 19 pair-fed rats were fed at 1900 hr. On day 20 +ZnPF rats were fed at 2000 hr and feed was removed from all rats at 2200 hr. All +ZnPF rats had consumed their diet by this time. The rats were then fasted overnight for 12 hr. For the second group, on each occasion the above regime was postponed by two hours. After the fast, rats were anesthetized lightly with diethyl ether and killed by decapitation. Trunk blood was collected in zinc-free, heparinized polystyrene tubes and immediately put on ice. Whole blood 2,3 DPG and ATP were measured using analytical kits obtained from Sigma Chemical Company (St. Louis, MO) (Procedures 35-UV and 366-UV). Assays were performed on whole blood in an attempt to preserve physiological concentrations of these metabolites in the erythrocytes.[15] Both procedures involve NADH-linked enzyme assays. The collected blood was precipitated promptly by addition to a trichloroacetic acid (TCA) solution. The procedure in the ATP kit is to add 1.00 mL of blood to 1.00 mL of cold 12% TCA. We found by using this procedure that recovery of added ATP was only about 70%. By mixing 0.35 mL of blood to a chilled solution of 1.00 mL of TCA plus 0.65 mL of water, the final concentration of TCA was maintained and ATP recovery improved to approximately 95%. The same precipitation method was used for 2,3 DPG analysis.

For determination of ATP, concentration standards were analyzed at the same time as samples. A standard curve was constructed and values for samples were determined by interpolation from this curve. One blood sample, from an ad libitum-fed rat, was spiked with ATP and analyzed concurrently with the samples, to determine recovery of ATP. The 2,3 DPG assay was performed in an analogous fashion. Recovery of ATP and 2,3 DPG from spiked samples was 94.2 ± 1.6% and 99.1 ± 1.1%, respectively. For these assays, all samples and standards were determined in duplicate.

Plasma Zn concentration was determined by atomic absorption spectrophotometry[16] after diluting the plasma 1:5 with deionized water. Hemoglobin was determined using an analytical kit obtained from Sigma Chemical Company (St. Louis, MO) (Procedure 525). All parameters were measured on each rat except for plasma zinc. Because the entire blood sample was used for 2,3 DPG, ATP, hematocrit, and hemoglobin determinations, plasma zinc values are for separate rats raised on the same dietary regime. The basal diet was dry ashed[17] and its Zn concentration determined by flame atomic absorption spectrophotometry.

Statistical analysis

Comparisons within each parameter were made by an analysis of variance followed by Tukey's studentized range test. The three different occasions that rats were raised were treated as replicates in the statistical model, and the two groups that the rats were divided into for the first two occasions were treated as blocks. The variability in the zinc deficient group was higher for some parameters. For these parameters, dietary treatments were compared in a pairwise manner using the model described above.

Results

Dietary Zn deficiency in rats caused a significant reduction in weight gain and plasma zinc concentration compared to pair-fed and ad libitum-fed controls. Zn deficiency resulted in a significantly elevated hematocrit but did not alter mean corpuscular hemoglobin concentration. The voluntary reduction in feed intake associated with dietary Zn deficiency caused an elevated blood hemoglobin concentration. Results are shown in *Table 1*.

The reduction of feed intake associated with dietary Zn deficiency caused a significantly elevated concentration of 2,3 DPG and a significantly decreased concentration of ATP in whole blood; however, per mL

Table 2 Effect of zinc deficiency on blood ATP and 2,3 DPG levels

	−Zn	+ZnPF	+ZnAL
2,3 DPG (μmol/mL blood)	3.93 ± 0.12[a]	3.88 ± 0.04[a]	3.62 ± 0.07[b]
2,3 DPG (μmol/mL RBC)	8.23 ± 0.21[a]	8.69 ± 0.10[a]	9.19 ± 0.13[b]
ATP (μmol/mL blood)	0.711 ± 0.018[a]	0.681 ± 0.026[a]	0.785 ± 0.018[b]
ATP (μmol/mL RBC)	1.49 ± 0.04[a]	1.52 ± 0.06[a]	1.99 ± 0.04[b]
2,3 DPG/ATP	5.54 ± 0.17[a]	5.75 ± 0.21[a]	4.62 ± 0.07[b]

Note: Mean ± SEM, $n = 8$. Values of a particular row having different superscript letters are significantly different ($P < 0.05$) by Tukey's test. For the statistical analysis of 2,3 DPG, the zinc deficient group had higher variability in these parameters, so paired comparisons were performed.

of erythrocytes, both concentrations were reduced significantly. A similar effect was observed when the concentrations of ATP and 2,3 DPG were expressed per mg of hemoglobin (data not shown). The reduction in feed intake also caused a significant elevation of the 2,3 DPG to ATP ratio in erythrocytes. Results are shown in *Table 2*.

Discussion

The hematological profile of Zn deficient rats using this experimental model has been described previously.[18] Feed restriction leads to microcytosis in rats[18] and mice[19]; this may be part of an effort to maintain a constant level of erythrocyte surface area.[20,21] Rapidly growing young rats are in a hematological state that has lower hemoglobin and hematocrit compared to adult levels. Given diets that contain levels of Fe and Cu adequate for maximal growth, weanling rats gradually increase blood hemoglobin and hematocrit until adult levels are reached.[22] The depressed growth seen in dietary zinc deficiency and feed restriction apparently accelerates this process; increased hemoglobin and hematocrit occur largely because of decreased tissue growth as opposed to enhanced erythropoiesis.[18] In the current experiment, the Zn deficient rats had the most depressed growth and the highest hematocrit. Blood ATP and 2,3 DPG concentrations are both thought to be regulated, in part, by blood hematocrit.[23]

Both 2,3 DPG and ATP, in addition to binding hemoglobin and modulating oxygen-carrying capacity,[23] have been proposed to affect the structure and function of the erythrocyte membrane. 2,3 DPG increases the lateral mobility of membrane glycoproteins,[11] decreases the mechanical stability and deformability of resealed erythrocyte ghosts,[24] decreases deformability of intact erythrocytes,[25] and increases the rate of dissociation of isolated membrane skeletal complexes.[26] Spectrin has been shown to have 2,3 DPG binding sites; 2,3 DPG binds to spectrin with approximately the same affinity that it binds to oxygenated hemoglobin.[27] 2,3 DPG inhibits spectrin-actin association in vitro both in the presence and absence of protein 4.1.[28] ATP increases the lateral mobility of glycoproteins in resealed erythrocyte ghosts[11] and increases the dissociation of isolated membrane skeletons.[26] ATP, as does 2,3 DPG, inhibits the association of spectrin and actin in vitro.[28] In vitro depletion of ATP is correlated with decreased levels of phosphatidylinositol biphosphate and increased or constant levels of phosphatidylinositol monophosphate[29,30]; phosphatidylinositol biphosphate may control the binding of protein 4.1 to the membrane-bound glycophorin C.[31,32] Depletion of ATP is also associated with altered levels of protein phosphorylation[33,34]; in vitro effects of the phosphorylation of several membrane skeleton proteins on membrane skeleton function have been described.[35,36] Depletion of erythrocyte ATP in vitro has been shown to result in shape change,[37] release of membrane vesicles,[38] an increase in osmotic fragility and a decreased deformability.[39]

The significant decrease in the concentrations of both major polyphosphates, 2,3 DPG and ATP, in erythrocytes from feed-restricted rats is suggestive of an altered biochemistry of the membrane skeleton. However, in zinc deficient rats, the relation between decreased erythrocyte 2,3 DPG and ATP concentrations and altered membrane function is unknown. Also, the possibility exists that the 2,3 DPG and ATP concentrations available to modify membrane function vary with the dietary treatments. Any further investigation must consider the possibility that significant decreases of erythrocyte membrane Zn[4,40] and polyamine[41] concentrations which also occur in Zn deficiency are part of a coordinate response to maintain cell membrane integrity. Finally, the elevated 2,3 DPG/ATP ratio in Zn deficiency and feed restriction predicts altered activity of specific kinases that use ATP as a substrate and may be inhibited by 2,3 DPG in vivo.[42,43]

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