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Erythrocyte deformability in zinc deficiency measured as a function of shear stress in the ektacytometer

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Erythrocyte deformability was investigated as a potential biochemical indicator of zinc status. Weanling, male Sprague-Dawley rats were fed ad libitum *modified AIN-93G diets containing 3 mg zinc/kg diet (−Zn;* n = 10*) for 6 weeks. Control rats were pair-fed (+ZnPF;* n = 10*) or fed* ad libitum *(+ZnAL;* n = 9*) diets containing 50 mg zinc/kg diet. Zinc deficiency significantly decreased plasma and tibia zinc concentration (*$P < 0.001$*). Erythrocyte deformability was measured on whole blood as a function of shear stress in the ektacytometer. Elongation index, the ratio of length to width of the diffraction pattern of deforming cells, was plotted against shear stress. Maximum elongation index and the initial slope were determined from a function used to fit the sigmoid curve. As analyzed by one-factor analysis of variance, maximum elongation index, a measure of average deformability of the cell population, was significantly depressed in the −Zn and +ZnPF groups as compared with the +ZnAL group (*$P < 0.05$*). The mean (±SEM) maximum elongation index for each of the three groups was −Zn, 0.55 ± 0.01; +ZnPF, 0.56 ± 0.01; +ZnAL, 0.59 ± 0.01. The initial slope of the curve, a measure of membrane deformability, was not altered by zinc deficiency. No effect of zinc deficiency was found for deformability of erythrocyte suspensions heated at 48°C for 6 min. Results of this study suggest that erythrocyte deformability would not be a useful functional indicator of zinc status.* (J. Nutr. Biochem. 9:457–463, 1998)
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Introduction

The detection of zinc deficiency has been difficult owing to a lack of a specific and sensitive biochemical indicator of zinc status.[1] We proposed that the ability of the red blood cell to undergo marked change in shape would be a sensitive functional indicator of zinc status. This critical physiological property of erythrocyte deformability reduces blood viscosity in large vessels, and allows erythrocytes to pass through the microcirculation for optimal delivery of oxygen despite their large diameter relative to that of capillaries.[2,3] Erythrocyte deformability is determined not only by internal viscosity and surface area to volume ratio but also by membrane viscoelastic properties.[4] Given the physiological function of zinc in the plasma membrane of mammalian cells,[5] it was hypothesized that red blood cell deformability would be altered by zinc deficiency.

During dietary zinc deficiency, the zinc concentration of the erythrocyte membrane is depressed in response to low extracellular zinc levels.[6] Dietary zinc deficiency in rats increases the osmotic fragility of erythrocytes,[7] but this is improved by the supplementation of dietary antioxidants.[8] Abnormalities reported in the erythrocyte membrane of severely zinc-deficient rats include increases in fluidity of the lipid bilayer and mobility of sialic acid residues on the cell surface.[9] Increased membrane protein mobility has been

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reported by some[10] but not all[11] investigators. Because most of the probe used to assess membrane protein mobility binds to the spectrin-actin complex, it has been suggested that spectrin structure and function might be altered in zinc deficiency.[10] This idea was further supported by findings of increased dephosphorylation of the skeletal membrane proteins, spectrin and actin, in erythrocyte membranes isolated from zinc-deficient rats.[12] The in vitro addition of zinc also modifies the phosphorylation patterns of isolated red blood cell membranes.[13] Normal cytoskeletal function is critical for cellular deformability, and the abnormal shape and/or increased fragility seen in elliptocytosis or spherocytosis is the result of absent or abnormal cytoskeletal proteins.[2,3] Thus, the changes that occur in the erythrocyte membrane during zinc deficiency might be predicted to influence deformability by altering membrane viscoelastic properties. The in vitro addition of zinc to human erythrocyte membranes has also been shown to alter the conformation of band 3,[14,15] which interacts with spectrin and ankyrin to anchor the cytoskeleton to the lipid bilayer.[2]

Pharmacological levels of zinc have been shown to reduce the number of irreversibly sickled cells in patients with sickle cell disease,[16,17] and in vitro addition of zinc to erythrocytes from these patients increases their filterability.[18,19] The rigidity of human erythrocytes artificially hardened by heating was returned to near normal values when incubated with increasing pharmacological concentrations of zinc.[20] Zinc deficiency of varying degrees has not been reported to influence erythrocyte deformability as assessed by filterability.[12,21] Unfortunately, the technique of filterability is not sensitive to altered deformability resulting from membrane damage.[22] The ektacytometer is an instrument that can detect small changes in red cell deformability and distinguish the contribution of membrane viscoelastic properties relative to the other cellular determinants of deformability.[23] In the only study that utilized the ektacytometer to examine erythrocyte deformability in severe zinc deficiency, a small but significant decrease in whole-cell deformability measured as a function of osmolality was reported, but this was mainly attributed to the accompanying decline in food intake.[24]

While previous studies have demonstrated minimal effects of zinc status on red blood cell deformability, the techniques used have limitations. In particular, the ektacytometer is more sensitive to changes in whole-cell deformability due to membrane alterations when measured as a function of shear stress rather than as a function of osmolality.[4] The objective of the current study was to investigate the influence of moderate zinc deficiency on erythrocyte deformability measured as a function of increasing shear stress in the ektacytometer. It was anticipated that this methodology would be more sensitive to changes in membrane viscoelastic properties than techniques previously employed.

Methods and materials

Experimental design

Thirty male, weanling (41 to 61 g) Sprague-Dawley rats (Charles River Laboratories, St. Constant, Quebec, Canada) were acclimatized for 2 days and then randomly assigned to one of three treatment groups ($n = 10$ per group). The rats were supplied with deionized, distilled water ad libitum and housed in individual suspended stainless steel cages at 22°C with 12-h periods of light and dark. A modified American Institute of Nutrition ’93G (AIN-93G) semipurified diet[25] was fed for 40 to 43 days. The composition of the basal diet is shown in *Table 1*. The treatment groups included (1) the moderately zinc-deficient group (−Zn) fed a diet formulated to contain 3 mg zinc/kg diet ad libitum; (2) the ad libitum-fed control group (+ZnAL) fed the same diet formulated to contain 50 mg zinc/kg diet; and (3) the pair-fed control group (+ZnPF) fed the diet containing 50 mg/kg zinc but individually pair-fed to the rats in the moderately zinc-deficient group. Daily food intakes were measured and the rats were weighed weekly.

Table 1 Composition of basal diet[1]

Ingredients	Amount (g/kg diet)
Spray-dried egg white	200
Corn starch	400.486
Dextrinized corn starch	132
Sucrose	100
Fiber (Solkafloc)	50
Soybean oil	70
Mineral premix[2]	35
Vitamin premix[3]	10
Choline bitartrate	2.5
tert-butyl hydroquinone	0.014

[1]To vary the zinc concentration in the diet, premixes of zinc carbonate were prepared in sucrose.
[2]Mineral premix supplied the following concentration of minerals in g/kg mix: calcium carbonate, anhydrous, 80; calcium phosphate, dibasic, 377; potassium sulfate, 46.6; potassium citrate, tripotassium, 37; magnesium oxide, 24; ferric citrate, 6.06; manganous carbonate, 0.63; cupric carbonate, 0.30; potassium iodate, 0.01; sodium selenate, anhydrous, 0.01367; ammonium paramolybdate, 4 hydrate, 0.00795; sodium metasilicate, 9 hydrate, 1.45; chromium potassium sulfate, 12 hydrate, 0.275; lithium chloride, 0.0174; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; ammonium vanadate, 0.0066.
[3]Vitamin premix supplied the following concentration of vitamins in g/kg mix: thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3; Ca pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.22; cyanocobalamin (0.1% in mannitol), 2.5; all trans-retinyl palmitate (250,000 IU/g), 1.6; all rac-α-tocopheryl acetate (250 IU/g), 30; cholecalciferol (400,000 IU/g), 0.25; vitamin K (10 mg phylloquinone/g dextrose), 7.5.

Blood samples were collected over days 40 to 43, and erythrocyte deformability was measured on the same day the blood was collected. On the morning of blood sampling, animals in the +ZnPF group were fed and food was removed from the −Zn and +ZnAL groups. One hour later, food was removed from the +ZnPF group. Prior to sample collection, the animals were anesthetized with methoxyflurane (Metofane). Blood samples, taken by cardiac puncture, were collected in heparinized polystyrene tubes for measurements of erythrocyte deformability and plasma zinc concentration or in Microtainer Brand Tubes with EDTA (Becton Dickinson, Rutherford, NJ USA) for complete blood counts. Samples were placed on ice. Following blood sampling, the animals were killed by decapitation and the tibias were collected. The tibias and plasma were stored at −20°C to be used for analysis of zinc concentration.

Flame atomic absorption spectrophotometry (model 4000, Perkin-Elmer, Norwalk, CT USA) was used to determine plasma, tibia, and dietary zinc concentration.[26] Prior to the measurement, plasma was diluted 1:4 with deionized, distilled water, tibia was digested by a wet ashing procedure,[27] and diet was digested by a

dry ashing procedure.[28] For each sample run, National Institute of Standards and Technology bovine serum or bovine liver was included as a standard reference material. Recovery for the bovine serum was 101.1%. Recovery for bovine liver was 102.8% for the tibia wet ashing procedure and 95.6% for the diet dry ashing procedure. The flame atomic absorption spectrophotometer was in absorbance mode for all readings. A linear regression equation, determined by six standard concentrations made in 0.1 mol/L ultrex-grade HNO_3, was used to calculate concentrations from the absorbance values.

A complete blood count was obtained by routine Coulter Counter techniques (Coulter Counter T660, Coulter Electronics, Hialeah, FL USA) if the volume of the blood sample obtained was adequate.

Erythrocyte deformability

The ektacytometer (model 152, Technicon Instruments, Tarrytown, NY USA) was used to perform erythrocyte deformability measurements. This instrument measures shear-induced elongation of erythrocytes. A cell suspension is placed in a viscometer consisting of a stationary and a movable cylinder that rotates to create a shear-stress field. A laser beam is passed through the suspension and a diffraction image is generated. Photometric measurement of the light intensity at two equidistant points from the centre of the diffraction pattern on each of the vertical and horizontal axes is used to quantitate erythrocyte deformability.[3,29] The elongation index (EI) was defined as

$$EI = (L - W)/(L + W)$$

where L and W represent length and width of the diffraction pattern. This parameter is used as a measure of erythrocyte deformability induced by a defined level of applied shear stress.[29,30]

Whole blood (37.5 μL) was suspended in 15.0 mL of solution containing 0.12 mmol/L polyvinylpyrrolidone (PVP), 6.34 mmol/L Na_2HPO_4, 2.00 mmol/L NaH_2PO_4, 6.15 mmol/L NaN_3, and 123.85 mmol/L NaCl (pH 7.35 ± 0.05) (PVP base solution). This solution was modified from that previously published[29,31] by increasing the percentage of PVP and NaCl to achieve the desired viscosity and osmolality of 22 mPa · s at 25°C and 290 ± 5 mmol/kg, respectively. The osmolality of this solution was measured by a freezing-point depression osmometer (model 3MO, Advanced Instruments Inc., Needham Hts., MA USA). The viscosity of the solution was measured using a cone and plate viscometer (model DV-111, Brookfield Engineering Laboratories, Stoughton, MA USA).

The whole-blood preparation was loaded into the stationary viscometer of the ektacytometer. The erythrocytes were exposed to increasing shear stress (0–29.8 Pa) as the rotation speed of the viscometer was increased.[29] Elongation index was plotted against shear stress on a linear recorder (model 8036, Linear). This measurement was done in duplicate from a single blood sample from each rat. Room temperature was recorded on each day of the experiment, as temperature affects the viscosity of the PVP base solution.[32]

To increase the sensitivity of the assay, erythrocytes were also heated to decrease membrane elasticity.[33] Whole blood was centrifuged at 1000*g* for 10 min at 4°C. Plasma and buffy coat were removed by aspiration. Erythrocytes were washed twice in phosphate-buffered saline and collected by centrifugation at 800*g* for 10 min at 4°C. The erythrocytes were then diluted 1:2 in phosphate-buffered saline, incubated at 48°C for 6 min[31] in a shaking water bath, and subsequently placed on ice. The erythrocyte suspension (37.5 μL) was suspended in 15.0 mL of the PVP base solution and erythrocyte deformability was measured as described above. Erythrocyte suspensions prepared in an identical manner but not heat-treated were also subjected to the assay.

A quality-control material was developed for the ektacytometer to ensure that there was no significant day-to-day variation in the instrument over the 4 days on which erythrocyte deformability was measured. Citrate-phosphate-dextrose solution (89.43 mmol/L trisodium citrate, 15.56 mmol/L citric acid monohydrate, 141.98 mmol/L dextrose monohydrate, and 16.09 mmol/L monobasic sodium phosphate) was added in a ratio of 1.4 mL to 10 mL whole rat blood[34] to preserve erythrocyte deformability over 4 days. Erythrocyte deformability was measured on this sample at the beginning and end of each day.

Utilizing the ektacytometer in the elongation index versus shear stress mode, two parameters were used to define erythrocyte deformability: (1) The maximum elongation index (EImax) provides an estimate of average deformability of the erythrocyte population.[29] The initial slope of elongation index plotted against shear stress is used to assess the contribution of membrane deformability to deformability of whole cells.[29]

Ten values for elongation index were plotted against the corresponding values for shear stress. Based on the sigmoidal shape of the curve, EImax was determined by fitting the data (Deltagraph Pro3, Deltapoint Inc., Monterey, CA USA) with the following function:

$$EI = \frac{EI_{max} \times \tau_s^n}{\tau_{s50}^n + \tau_s^n}$$

where EI = elongation index; EI_{max} = maximum elongation index (EImax); τ_{s50} = the shear stress producing 50% of maximum elongation index; τ_s = shear stress and n = sigmoidicity. Because of the sigmoidal nature of the curve, maximum slope was calculated for the linear portion between the values of 0.05 and 0.1 for elongation index. This provided a value close to expected initial slope in a hyperbolic curve.

Statistical analysis

A paired *t*-test was used to compare deformability parameters for heat-treated erythrocyte suspensions against those obtained from untreated erythrocyte suspensions (Statview, Abacus Concepts Inc., Berkeley, CA USA). Other data were analyzed using one-way ANOVA followed by Fisher's Protected LSD (SuperANOVA, Abacus Concepts Inc., Berkeley, CA USA).

Source of chemicals

Methoxyflurane (Metofane) was supplied by Janssen Pharmaceutica (Mississauga, ON, Canada). Sigma Chemical Co. (St. Louis, MO USA) supplied the polyvinylpyrrolidone, NaN_3, and citric acid monohydrate. Na_2HPO_4, NaH_2PO_4, NaCl, and trisodium citrate were obtained from BDH Inc. (Toronto, Canada). ICN Inc. (Mississauga, ON, Canada) supplied dextrose monohydrate. PDI Bioscience (Montreal, Canada) supplied HNO_3, Ultrex II. The National Institute of Standards and Technology bovine serum or bovine liver was provided by the U.S. Department of Commerce, National Bureau of Standards (Gaithersburg, MO USA).

Results

One rat from the +ZnAL group was excluded from the study owing to blood loss from a broken toenail.

The zinc-deficient diet was found to contain 3.7 mg Zn/kg diet, and the control diet contained 54.6 mg Zn/kg diet. The zinc-deficient diet resulted in a moderate zinc deficiency as demonstrated by depressed plasma and tibia zinc concentration as compared to that of pair-fed or ad

Table 2 Effect of dietary zinc on zinc status

	Experimental group[1]		
	−Zn[2]	+ZnPF[3]	+ZnAL[4]
Total weight gain (g)	173.1 ± 3.9—a	183.0 ± 5.7—a	333.1 ± 11.0—b
Total food intake (g)	518.2 ± 10.4—a	510.9 ± 10.3—a	814.8 ± 21.6—b
Food efficiency[5]	0.333 ± 0.004—a	0.357 ± 0.007—b	0.408 ± 0.008—c
Plasma zinc (μmol/L)	1.99 ± 0.31—a	15.45 ± 1.22—b	23.10 ± 1.84—c
Tibia zinc (μmol/g dry weight)	0.64 ± 0.03—a	4.67 ± 0.12—b	5.10 ± 0.09—c

[1]Results expressed as mean ± SEM. Statistical analysis was a one-factor ANOVA followed by Fisher's Protected LSD. Means in the same row with different letters (a, b, and c) are significantly different ($P < 0.001$).
[2]Weanling rats fed a diet formulated to contain 3 mg Zn/kg diet ad libitum for 6 weeks ($n = 10$ with the exception of plasma [$n = 7$]).
[3]Weanling rats pair-fed a diet formulated to contain 50 mg Zn/kg diet for 6 weeks ($n = 10$).
[4]Weanling rats fed a diet formulated to contain 50 mg Zn/kg diet ad libitum for 6 weeks ($n = 9$).
[5]Weight gain/food intake.

libitum-fed control groups (*Table 2*). Both plasma and tibia zinc concentrations were slightly but significantly decreased in the +ZnPF group as compared to the +ZnAL group values. Food intake and weight gain were significantly depressed in the −Zn group as compared to the +ZnAL group (*Table 2*). Food efficiency was depressed in the −Zn group compared to the +ZnAL group with the +ZnPF group having an intermediate value (*Table 2*). Dermatological lesions were present in the zinc-deficient group only.

The hematological profile for the three groups is shown in *Table 3*. The −Zn group had a significantly increased red cell distribution width (a measure of variability of erythrocyte volume) over the other two groups. Hemoglobin and mean corpuscular hemoglobin were significantly decreased in the −Zn and +ZnPF groups as compared to the +ZnAL group. The −Zn group had significantly lower mean corpuscular hemoglobin concentration than did the +ZnAL group, with the +ZnPF group having an intermediate value. No differences among groups were found for red blood cell count, hematocrit, mean corpuscular volume, or reticulocyte count. There was an outlier value of 1474×10^9 reticulocytes per liter for reticulocyte count in the −Zn group. Without this value, the mean ± SEM of the −Zn group is $297 \times 10^9 \pm 115 \times 10^9$ reticulocytes/L.

Figure 1 shows elongation index plotted as a function of shear stress for untreated and heat-treated erythrocyte suspensions from a representative control (+ZnAL) rat. Room temperature during the 4 days of deformability measurements ranged from 23°C to 24°C. Mean (±SEM) EImax and initial slope measured on the quality-control material were 0.54 ± 0.01 and 0.018 ± 0.001, respectively, over this period. *Table 4* shows the effect of dietary zinc deficiency on erythrocyte deformability. Maximum elongation index (EImax) for whole blood from the +ZnAL group was significantly higher than that of the −Zn and +ZnPF groups. The initial slope was not altered by experimental treatment. Deformability of erythrocyte suspensions and heat-treated erythrocyte suspensions was not significantly altered by zinc deficiency or reduced food intake (*Table 4*).

Paired *t*-test analysis on pooled data from all experimental groups demonstrated a significant decrease in EImax and initial slope when RBC suspensions were heat-treated ($P < 0.05$). The mean (±SEM) EImax for the untreated erythrocyte suspensions was 0.54 ± 0.01 as compared with 0.46 ± 0.02 for the heat-treated erythrocyte suspensions. The mean (±SEM) slope for the erythrocyte suspensions and for the heat-treated erythrocyte suspensions was 0.015 ± 0.0004 and 0.007 ± 0.001, respectively.

Table 3 Influence of dietary zinc deficiency on hematological profile

	Experimental group[1]		
	−Zn[2]	+ZnPF[3]	+ZnAL[4]
Red blood cell count ($\times 10^{12}$/L)	6.38 ± 0.31—a	6.39 ± 0.18—a	6.50 ± 0.17—a
Hemoglobin (g/L)	123.0 ± 2.0—a	126.7 ± 2.5—a	136.1 ± 3.4—b
Hematocrit (L/L)	0.36 ± 0.01—a	0.37 ± 0.01—a	0.39 ± 0.01—a
Mean corpuscular volume (fL)	57.0 ± 2.4—a	57.5 ± 0.7—a	59.5 ± 0.5—a
Mean corpuscular hemoglobin (pg)	19.4 ± 0.8—a	19.8 ± 0.2—a	21.0 ± 0.2—b
Mean corpuscular hemoglobin concentration (g/L)	340.8 ± 0.9—a	345.1 ± 2.5—ab	352.0 ± 2.7—b
Red cell distribution width (%)	13.7 ± 1.1—a	10.2 ± 0.3—b	10.5 ± 0.5—b
Reticulocyte count (%)	10.1 ± 5.7—a	3.1 ± 0.7—a	4.0 ± 0.3—a
Reticulocyte count ($\times 10^9$/L)	592 ± 305—a	192 ± 36—a	260 ± 21—a

[1]Results expressed as mean ± SEM. Statistical analysis was a one-factor ANOVA followed by Fisher's Protected LSD. Means with different letters (a, b, and c) in the same row are significantly different ($P < 0.05$).
[2]Weanling rats fed a diet formulated to contain 3 mg Zn/kg diet ad libitum for 6 weeks ($n = 4$).
[3]Weanling rats pair-fed a diet formulated to contain 50 mg Zn/kg diet for 6 weeks ($n = 7$).
[4]Weanling rats fed a diet formulated to contain 50 mg Zn/kg diet ad libitum for 6 weeks ($n = 7$).

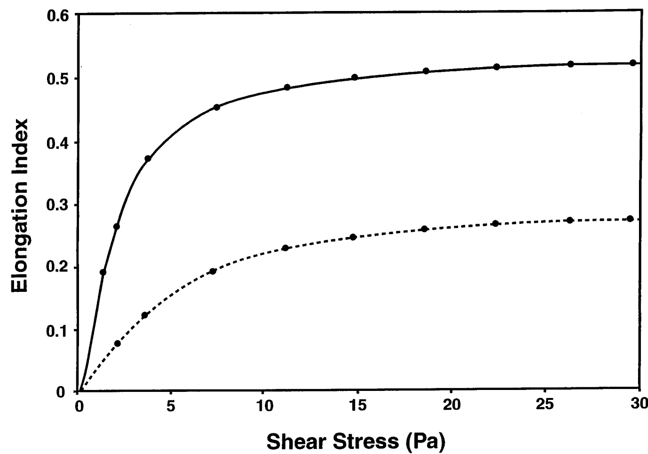

Figure 1 Elongation index plotted as a function of shear stress for untreated (———) and heat-treated (— — —) red blood cell suspensions from a representative control (+ZnAL) rat.

Discussion

The diagnosis of zinc deficiency remains an important problem in human health, yet assessment is difficult.[1] Although many biochemical indicators of zinc status have been proposed, none has proven to be a singularly reliable test.[1] Most investigators advise the use of a combination of both static and functional indicators.[1] Although erythrocyte zinc concentration does not consistently decline in zinc deficiency,[35–38] erythrocyte membrane zinc is depleted in experimental animals.[6] Measurements of zinc-related functions in the erythrocyte or its membrane have been examined because of the accessibility of this cell in the human population. Ruz et al.[38] showed decreased activity of erythrocyte membrane alkaline phosphatase activity during experimental zinc deficiency in young men, and Samman et al.[39] reported increased activity of this enzyme in erythrocytes of healthy males supplemented with 50 mg zinc per day for 4 weeks. Fischer and Bettger[40] examined the relationship between zinc status and the zinc efflux rate from erythrocytes incubated in the presence of the chelator, *o*-phenanthroline, but found inadequate sensitivity for developing this as a functional index of zinc status.

We proposed that erythrocyte deformability measured as a function of shear stress in the ektacytometer would be a sensitive index that could be included in a battery of tests used in the diagnosis of zinc deficiency. The rats utilized in our study were moderately zinc-deficient as shown by decreased plasma and tibia zinc concentrations and the presence of dermatological lesions. The hematological profile of both zinc-deficient and food-restricted controls showed only minor deviations from normal, and there was no evidence of the pronounced increase in hemoglobin and hematocrit that is present in severely zinc-deficient rats.[41]

Erythrocytes were subjected to increasing shear stress in the ektacytometer, and maximum elongation index and the initial slope of elongation index plotted against shear stress were monitored to provide estimates of the average deformability of the red cell population and the contribution of membrane deformability, respectively. There was no effect of zinc deficiency on either of these parameters when measured on whole blood or on red blood cell suspensions, although there was a small depression in the maximum elongation index of whole-blood samples that can be attributed to the food restriction associated with the zinc deficiency. Incubating the erythrocyte suspensions at 48°C depressed cell deformability by decreasing membrane deformability, as would be predicted from the reported reduction in elasticity associated with this treatment.[33] However, the effect of the heat treatment did not differ among the three experimental groups.

Our findings suggest that there is no marked change in the ability of the erythrocyte to undergo shape change in a zinc-deficient state. This is in agreement with the results of previous studies that have explored this question with less sensitive techniques.[12,21,24] The decline in deformability that occurred with heat treatment of erythrocyte suspensions demonstrates that the technique we employed can detect alterations in cell deformability caused by gross changes in

Table 4 Erythrocyte deformability measured as a function of increasing shear stress in dietary zinc deficiency

	Experimental group[1]		
	−Zn[2]	+ZnPF[3]	+ZnAL[4]
Elmax[5]			
Whole blood	0.55 ± 0.01—a	0.56 ± 0.01—a	0.59 ± 0.01—b
RBC suspensions	0.53 ± 0.01—a	0.53 ± 0.01—a	0.55 ± 0.01—a
Heat-treated RBC suspensions[6]	0.46 ± 0.03—a	0.44 ± 0.03—a	0.47 ± 0.03—a
Slope[7]			
Whole blood	0.017 ± 0.002—a	0.017 ± 0.001—a	0.021 ± 0.002—a
RBC suspensions	0.016 ± 0.001—a	0.015 ± 0.001—a	0.016 ± 0.0003—a
Heat-treated RBC suspensions	0.007 ± 0.001—a	0.007 ± 0.001—a	0.007 ± 0.001—a

[1]Results expressed as mean ± SEM. Statistical analysis was a one-factor ANOVA. Means with different letters (a, b, and c) are significantly different ($P < 0.05$).
[2]Weanling rats fed a diet formulated to contain 3 mg Zn/kg diet ad libitum for 6 weeks ($n = 10$).
[3]Weanling rats pair-fed a diet formulated to contain 50 mg Zn/kg diet for 6 weeks ($n = 10$).
[4]Weanling rats fed a diet formulated to contain 50 mg Zn/kg diet ad libitum for 6 weeks ($n = 9$).
[5]The maximum elongation index.
[6]RBC suspensions were heated at 48°C for 6 min.
[7]The initial slope of elongation index plotted against shear stress.

membrane flexibility. Previous measurements made under similar conditions in the ektacytometer have also shown maximum elongation and initial slope to be depressed by 10% to 30% in hereditary spherocytosis.[42] This disorder is characterized by abnormalities in one or more cytoskeletal proteins.[2,43] We had predicted that erythrocyte deformability would be impaired on the basis of previous studies suggesting that zinc deficiency causes abnormalities in cytoskeletal proteins or in proteins important for anchoring the cytoskeleton to the lipid bilayer.[10,12,14,15] The findings of the current study indirectly suggest that cytoskeletal regulation of erythrocyte membrane deformation is normal, at least in moderate zinc deficiency. Other in vivo studies describing abnormalities in components of the cytoskeleton have more often studied severe zinc deficiency.[10,12] Avery and Bettger[44] have described structural and functional characteristics of spectrin in dietary zinc deficiency, and they have reported no alteration in erythrocyte membrane spectrin content, oligomeric form, or extractibility.

Another explanation for our findings is that moderate zinc deficiency exerts a subtle influence on erythrocyte deformability that could not be detected by the whole-cell assay. A more definitive answer should be obtained by utilizing resealed erythrocyte membranes in place of whole cells in the ektacytometer. The use of ghosts eliminates internal viscosity and surface area to volume ratio as contributors to erythrocyte deformation and allows for direct measurement of the physical properties of the erythrocyte membrane.[45,46] Future studies should also examine the effect of zinc deficiency on erythrocyte stability, the maximum extent of deformation that a red cell can undergo beyond which it cannot recover its initial shape.[30] This can be measured in the ektacytometer as the time required to fragment cells or ghosts under high shear stress. The latter technique has been shown to detect membrane abnormalities that are not evident with other measurements of deformability in the ektacytometer.[46] While further studies on membrane deformability will assist in understanding the functional effects of zinc on the membrane, the need to prepare erythrocyte ghosts would limit the practicality of such a biochemical tool for assessing zinc status in clinical laboratories.

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