

Zinc Deficiency Affects DNA Damage, Oxidative Stress, Antioxidant Defenses, and DNA Repair in Rats^{1–3}

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Abstract

Approximately 12% of Americans do not consume the Estimated Average Requirement for zinc and could be at risk for marginal zinc deficiency. Zinc is an essential component of numerous proteins involved in the defense against oxidative stress and DNA damage repair. Studies *in vitro* have shown that zinc depletion causes DNA damage. We hypothesized that zinc deficiency *in vivo* causes DNA damage through increases in oxidative stress and impairments in DNA repair. Sprague-Dawley rats were fed zinc-adequate (ZA; 30 mg Zn/kg) or severely zinc-deficient (ZD; <1 mg Zn/kg) diets or were pair-fed zinc-adequate diet to match the mean feed intake of ZD rats for 3 wk. After zinc depletion, rats were repleted with a ZA diet for 10 d. In addition, zinc-adequate (MZA 30 mg Zn/kg) or marginally zinc-deficient (MZD; 6 mg Zn/kg) diets were given to different groups of rats for 6 wk. Severe zinc depletion caused more DNA damage in peripheral blood cells than in the ZA group and this was normalized by zinc repletion. We also detected impairments in DNA repair, such as compromised p53 DNA binding and differential activation of the base excision repair proteins 8-oxoguanine glycosylase and poly ADP ribose polymerase. Importantly, MZD rats also had more DNA damage and higher plasma F₂-isoprostane concentrations than MZA rats and had impairments in DNA repair functions. However, plasma antioxidant concentrations and erythrocyte superoxide dismutase activity were not affected by zinc depletion. These results suggest interactions among zinc deficiency, DNA integrity, oxidative stress, and DNA repair and suggested a role for zinc in maintaining DNA integrity. *J. Nutr.* 139: 1626–1631, 2009.

Introduction

Zinc deficiency is an important worldwide public health problem with ~2 billion people who do not ingest adequate amounts of zinc (1). Data from NHANES 2001–2002 show that ~12% Americans do not consume the Estimated Average Requirement for zinc; thus, a large proportion of the U.S. population could be at risk for marginal zinc deficiency (2). Epidemiological studies reveal associations between low circulating zinc concentrations and increased risk of cancer (3,4). However, the mechanisms by which zinc deficiency increase the risk of cancer are still unclear and understudied. Zinc is an important element in numerous proteins and plays a pivotal role in several essential cell functions such as cell proliferation and apoptosis, defense against free radicals, and DNA damage

repair. For instance, CuZn superoxide dismutase (SOD)⁶ is an important first-line defense enzyme against oxygen radical species and p53 is an important zinc-containing transcription factor that plays an essential role in the DNA damage response. Low cellular zinc may increase oxidative stress, impair DNA binding activity of p53, and interfere with its functions in DNA repair (5,6). Thus, several different mechanisms may be involved in processes leading to impaired DNA integrity with zinc deficiency *in vivo*. Zinc deficiency may increase oxidative stress that directly causes DNA damage and may impair DNA damage repair responses (7).

Although increasing evidence suggests that zinc has antioxidant properties and protects tissue from oxidative damage (5,8–15), many of these studies have only used severe zinc-depletion protocols that obstruct growth and development and have little physiological relevance in the general population. In contrast, marginal zinc deficiency is more physiologically relevant to

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³ Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.

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⁶ Abbreviations used: AA, arachidonic acid; BER, base excision repair; FRAP, ferric reducing ability of plasma assay; MZA, zinc-adequate group for marginal zinc deficiency study; MZD, marginally zinc-deficient group; OGG1, 8-oxoguanine glycosylase; PARP, poly ADP ribose polymerase; PF, pair-fed group; SOD, superoxide dismutase; SSB, single-strand break; ZA, zinc-adequate group; ZD, severely zinc-deficient group; ZnRe, zinc repletion group.

human zinc deficiency (16), yet little is known about its effect on oxidative stress and DNA damage.

Our current rat study examined the *in vivo* interactions among zinc deficiency, DNA damage, oxidative stress, antioxidant defenses, and DNA repair in both severely and marginally zinc-depleted rats. We hypothesized that alterations in zinc status would affect DNA integrity by altering oxidative stress, antioxidant defenses, and DNA repair functions. Previous studies conducted in our laboratory have demonstrated that severe zinc depletion increases oxidative stress biomarkers in rat plasma (15). In the current study, we further assessed DNA damage and DNA repair proteins in zinc-depleted rats and, importantly, added a zinc repletion stage to test whether these deleterious effects are reversible. Second, we used a physiologically relevant, marginally zinc-depleted rat model and investigated the effects of marginal zinc deficiency on DNA integrity, oxidative stress, and DNA repair. Therefore, our study is one of the first to explore the effects of marginal zinc deficiency on DNA integrity *in vivo* and may shed light on human trials exploring the possible deleterious effects of marginal zinc deficiency in humans.

Materials and Methods

Rats and diets. The rat protocol was approved by Oregon State University's (Corvallis, OR) Institutional Laboratory Animal Care and Use Committee. Male Sprague-Dawley rats from Charles River were acclimated for 1 wk to the temperature- and humidity-controlled environment with a 12-h-dark/light cycle. The rats for the severe zinc deficiency study were maintained in stainless steel suspended cages and the rats for the marginal zinc deficiency study were maintained in polycarbonate cages. Diets were based on modified AIN-93G rodent diets (17) for growing rats or AIN-93M diets (17) for sexually mature rats, formulated with egg white rather than casein and with zinc provided as zinc carbonate (Dyets). Deionized water was provided as drinking water.

Severe zinc deficiency study. Rats (10/group, 3 wk old, ~50 g) were randomly assigned to 3 dietary treatments: zinc-adequate diet (ZA group; 30 mg Zn/kg), severely zinc-deficient diet (ZD group; <1 mg Zn/kg), or pair-fed zinc-adequate diet (PF group; 30 mg Zn/kg) to match the mean feed intake in the ZD rats. Ten rats fed the ZD diet for 21 d were switched to the zinc-adequate diet for up to 10 d for the zinc repletion group (ZnRe). Diet intakes and body weights were measured daily. Rats were killed following anesthesia with isoflurane overdose (1–5%; Henry Schein).

Marginal zinc deficiency study. Rats (12/group, 5 wk old, ~110 g) were randomly assigned to 1 of 2 dietary treatments: zinc-adequate diet (MZA group; 30 mg Zn/kg) or marginally zinc-deficient diet (MZD group; 6 mg Zn/kg) for 42 d. Diet intakes and body weights were measured twice every week. Rats were killed following anesthesia with isoflurane overdose (1–5%; Henry Schein).

Tissue and blood collection. Blood samples were collected by cardiac puncture into trace element-free vials containing EDTA. Plasma and erythrocytes was separated immediately and frozen at -80°C until analysis. Samples of liver were dissected and immediately snap frozen at -80°C until analysis.

Zinc analysis. Zinc concentrations were measured by inductively coupled plasma-optical emission spectrometry (Teledyne Leeman Labs) as described previously (15,18).

DNA damage. Single-strand breaks (SSB) in peripheral blood cells were determined by alkali single-cell gel electrophoresis (Comet assay) as described by Singh et al. (19). Images of 100 randomly selected nuclei

from each rat were analyzed for tail moments. Results are presented as the fold of the tail moments of the ZA or MZA group.

Oxidative stress. Plasma F_2 -isoprostanes were measured as an index of lipid peroxidation and indicator of oxidative stress *in vivo*. The sum of various F_2 -isoprostanes with the appropriate mass:charge ratio and fragmentation characteristics and arachidonic acid (AA) were measured in plasma as previously described (20) using HPLC (Shimadzu HPLC system) and multiple reaction monitoring on an Applied Biosystems/MDS Sciex API 3000 triple quadrupole mass spectrometer.

Total antioxidant capacity. The ferric reducing ability of plasma (FRAP) was measured at 550 nm on a microplate reader (Spectramax 190; Molecular Devices) as previously described (21). Plasma α -tocopherol and ascorbic concentrations were measured by HPLC-ECD as described (22–24).

Erythrocyte SOD activity. SOD activity was determined by the xanthine oxidase-cytochrome c method according to McCord and Fridovich (25) and L'Abbé et al. (26). Hemoglobin concentration in cell lysates was measured by Drabkin's method (27).

Western analysis of DNA repair proteins. Proteins (30 $\mu\text{g}/\text{lane}$) were separated by SDS-PAGE on a 4–12% bis-Tris gel (Invitrogen) and transferred to nitrocellulose membrane (Bio-Rad). The primary antibodies used for detection were mouse anti-p53 (Calbiochem), mouse anti-poly ADP ribose polymerase (PARP; BD pharmingen), rabbit anti-8-oxoguanine glycosylase (OGG1; Novus Biologicals), and mouse anti- β -actin (Sigma). Bound antibodies were detected using either goat anti-mouse IgG-horseradish peroxidase or goat anti-rabbit IgG-horseradish peroxidase (Santa Cruz Biotechnology) and developed with SuperSignal West Femto chemiluminescent substrate (Pierce). Images were acquired on an Alpha Innotech photodocumentation system and analyzed using Image J 1.37v software (NIH).

Electrophoretic mobility shift assay. p53 DNA binding activity was assessed by electrophoretic mobility shift assay using the p53 IRDye 700 Infrared Dye Labeled Oligonucleotides (LiCOR Biosciences) as previously described (28). Briefly, nuclear extract were mixed with p53 oligo IRDye 700. Infrared Dye was incubated at room temperature for 30 min. For specific competitor reactions, the sample was incubated with unlabeled p53 oligo before the addition of 50 fmol of labeled probe. Reaction mixture was separated on a 6% acrylamide gel imaged and quantified.

Statistical analysis. Statistical analysis was performed with the use of PRISM (version 4.0; GraphPad Software). One-way ANOVA were used for comparisons among the 4 dietary treatments with Tukey's post hoc test when appropriate. Student's *t* test was used for comparisons between MZD and MZA groups. Equal variances among groups were tested by Bartlett's test and logarithmic data transformation was performed in cases of unequal variance. Differences were considered significant at $P < 0.05$. All data are reported as mean \pm SEM unless otherwise indicated.

Results

Body and organ weights.

Severe dietary zinc restriction (<1 mg Zn/kg) resulted in anorexia and lower growth rates in the ZD rats than in the ZA and PF rats (data not shown), which also differed from one another. In addition, the ZD rats exhibited other signs of zinc deficiency, including loss of hair, decreased activity, and increased agitation. At the end of zinc depletion, body weights in ZD rats (110.8 ± 3.5 g) were less than in PF rats (136.6 ± 2.3 g), which were less than in ZA rats (226.8 ± 7.5 g) ($P < 0.05$). Liver and spleen weights similarly differed among all 3 groups ($P < 0.05$; data not shown). Repletion of the ZD rats with zinc-adequate diet for 10 d partially restored body weight in the

ZnRe group (202.0 ± 4.9 g), but it remained 31.7% lower than in the ZA group (291.6 ± 10.0 g) ($P < 0.05$). However, the growth rate during zinc repletion (9.41 ± 0.33 g body weight/d) was >8.5 times that during zinc depletion (1.08 ± 0.04 g body weight/d) ($P < 0.05$) and significantly greater than the growth rate of the ZA group (7.20 ± 0.32 g/d) ($P < 0.05$). Liver and spleen weights were also restored by zinc repletion (data not shown). Marginal zinc depletion also did not affect body and organ weights, as expected. At the end of the study, the body weight was 360.3 ± 12.8 g in the MZA group and 380.6 ± 8.4 g in the MZD group.

Tissue zinc concentrations.

Hepatic zinc concentrations were lower in the ZD rats (0.53 ± 0.03 $\mu\text{mol/g}$) than in the ZA rats (0.79 ± 0.09 $\mu\text{mol/g}$) ($P < 0.001$) and tended to be lower than in the PF rats (0.64 ± 0.03 $\mu\text{mol/g}$) ($P = 0.07$). Hepatic zinc concentrations also differed between ZA and PF rats ($P < 0.05$). These data in combination with the physiological alterations in the ZD rats confirmed that the rats fed the severely zinc-deficient diet developed zinc deficiency. The 10-d zinc repletion period increased hepatic zinc concentrations in the ZnRe rats (0.72 ± 0.03 $\mu\text{mol/g}$) to the control levels, suggesting that the rats achieved zinc-adequate status following the repletion period. The MZD rats also had lower hepatic zinc concentrations (0.66 ± 0.03 $\mu\text{mol/g}$) than the MZA rats (0.75 ± 0.02 $\mu\text{mol/g}$) ($P < 0.05$), confirming altered zinc status in the marginal zinc depletion model.

DNA damage in peripheral blood cells.

The mean tail moment of the ZD group was 1.3-fold that of the PF rats (Fig. 1) ($P < 0.05$), indicating an increase in SSB with severe zinc deficiency. The tail moment of the PF group did not differ from the ZA group. The 10-d zinc repletion reduced the tail moment to that of the PF and ZA controls, suggesting that DNA damage was reversible with zinc repletion.

The mean tail moment of the MZD group was 1.2-fold that of the MZA group ($P < 0.05$), indicating that marginal zinc deficiency is sufficient to significantly increase DNA damage (data not shown).

Oxidative stress in plasma.

Plasma AA concentrations, the precursor of F_2 -isoprostanes, a biomarker for oxidative stress, were significantly lower in the ZD rats and their PF rat controls than in the ZA rats ($P < 0.05$; data not shown). When plasma F_2 -isoprostane concentrations were normalized to AA concentrations, the concentrations of

plasma 15-series and 5-series F_2 -isoprostanes were greater in the ZD group than in the ZA groups (Fig. 2A,B; $P < 0.05$). After zinc repletion, the concentrations of plasma F_2 -isoprostanes did not differ between the ZnRe and ZA groups (Fig. 2A,B; $P > 0.05$). However, plasma F_2 -isoprostanes did not differ between the PF and ZD groups. It is likely that the severe food restriction in itself is a marked stress on the rats that could induce high oxidative stress (Fig. 3A); at the end of zinc depletion on d 20, feed intake in the PF group was 47% of that of the ZA group. Another possible explanation is that food restriction resulted in low zinc intake in the PF rats (Fig. 3B); at the end of zinc depletion on d 20, zinc intake in the PF group was 46% of that of the ZA group. This restricted zinc intake may effectively cause a marginal zinc deficiency. Because the hepatic zinc concentrations were 20% lower in the PF (0.64 ± 0.03 $\mu\text{mol/g}$) than in the ZA groups (0.79 ± 0.09 $\mu\text{mol/g}$) ($P < 0.05$), it further supports the concept that zinc status was compromised in the PF rats.

Importantly, the MZD rats also had higher plasma 15- and 5-series F_2 -isoprostane concentrations (15-series F_2 -isoPs, 5.23 ± 0.29 $\text{pg}/\mu\text{g AA}$; 5-series F_2 -isoPs, 10.07 ± 0.78 $\text{pg}/\mu\text{g AA}$) than the MZA rats (15-series F_2 -isoPs, 4.06 ± 0.24 $\text{pg}/\mu\text{g AA}$; 5-series F_2 -isoPs, 7.21 ± 0.40 $\text{pg}/\mu\text{g AA}$) ($P < 0.01$), yet plasma AA concentrations were not affected by marginal zinc depletion (data not shown).

Antioxidant defenses.

FRAP, vitamin C, vitamin E, and erythrocyte SOD. The ZD group had lower plasma FRAP values and AA and α -tocopherol concentrations than the ZA group ($P < 0.05$) but not compared with the PF group; dietary zinc repletion restored these to control levels (Table 1). Hepatic α -tocopherol concentrations in the ZD group were lower than in the ZA and PF groups (Table 1; $P < 0.05$), similar to our previous findings (15).

Antioxidant status did not differ between the MZD and MZA groups (Supplemental Table 1), suggesting that antioxi-

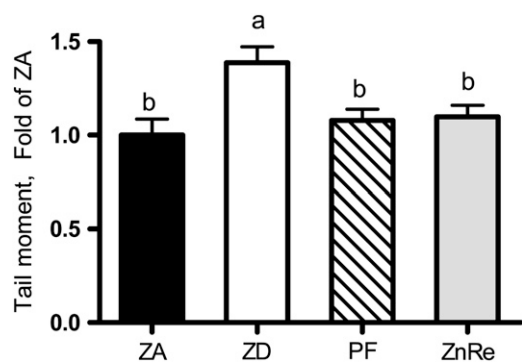


FIGURE 1 Effects of dietary zinc status on DNA SSB in rat peripheral blood cells. Values are means \pm SEM, $n = 10$. Means without a common letter differ, $P < 0.05$.

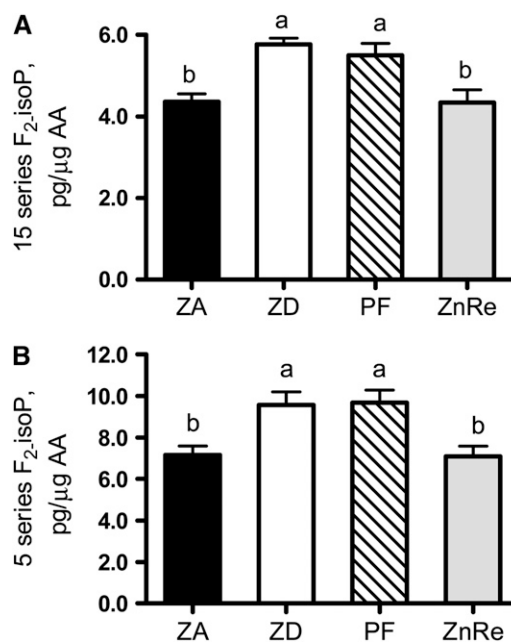


FIGURE 2 Effects of dietary zinc status on plasma 15 series F_2 -isoprostanes (IsoP) (A) and 5 series F_2 -isoprostanes (B) in rats. Values are means \pm SEM, $n = 10$. Means without a common letter differ, $P < 0.05$.

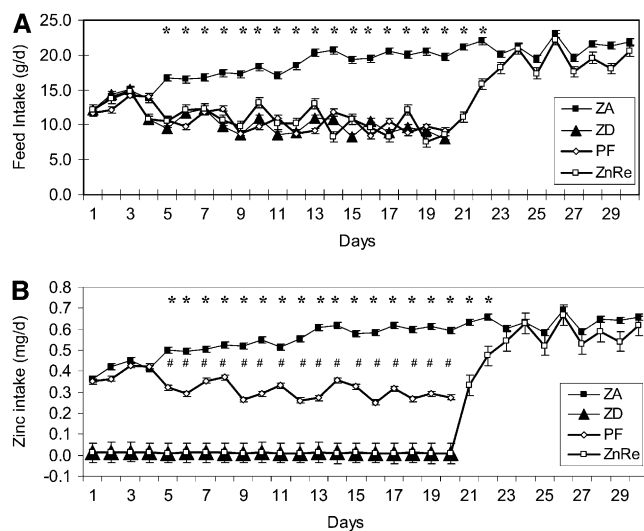


FIGURE 3 Effect of dietary zinc status on feed intake (A) and zinc intake (B) in rats fed a ZD, PF, or ZA diet for 21 d, and in ZnRe rats fed a ZD diet for 21 d and then a ZA diet for 10 d. Values are means \pm SEM, $n = 10$. *ZA differs from all other groups, $P < 0.05$. #PF differs from the ZD and ZnRe, $P < 0.05$.

dant defenses are inadequate to prevent the oxidative stress and DNA damage due to marginal zinc deficiency.

Erythrocyte SOD is comprised of cytosolic CuZnSOD, an important zinc-containing antioxidant defense enzyme in the circulation. Erythrocyte SOD activities were not altered with either severe (Table 1) or marginal zinc deficiencies (Supplemental Table 1).

Zinc-containing DNA repair proteins in livers. Hepatic OGG1 protein levels were substantially higher in the ZD group than in the ZA and PF groups, which did not differ from one another (Fig. 4A; $P < 0.05$). Zinc repletion reduced OGG1 levels in the ZnRe group did not differ from the ZA and PF groups. The MZD group also had higher OGG1 protein levels than the MZA group (Fig. 4D; $P < 0.05$). However, hepatic PARP protein levels did not differ among the ZD, ZA, PF, and ZnRe groups (Fig. 4B) or between the MZD and MZA groups (Fig. 4E).

The ZD group had higher p53 protein levels in livers than the PF and ZA groups (Fig. 4C; $P < 0.05$) and zinc repletion reduced p53 protein to control levels. However, DNA binding activity of p53 in liver nuclear extract was unchanged (data not shown), suggesting the functional activity of p53 might be compromised. The MZD group did not have altered p53 protein levels in liver

(Fig. 4F) and DNA binding activity of p53 was unchanged (data not shown), despite this group having more DNA damage than the MZD group. Therefore, DNA repair pathways may be impaired with severe and marginal zinc deficiencies.

Discussion

This study shows that both severe and marginal zinc deficiencies *in vivo* increase oxidative stress, impair DNA integrity, and increase DNA damage in rat peripheral blood cells. Concomitant with increases in DNA damage are impaired DNA repair functions with zinc deficiency. Importantly, DNA damage and oxidative stress biomarkers are reversed upon zinc repletion. This study highlights the importance of zinc in the maintenance of DNA integrity and points out that even marginal zinc deficiency has deleterious consequences and may increase the risk for DNA damage.

The mechanisms by which zinc deficiency affects DNA damage are unclear. We have previously postulated that increased DNA damage with zinc deficiency is a multi-factorial process involving both perturbations in oxidative stress and compromised DNA repair (7). Perturbations in oxidative stress with zinc deficiency may be attributed to the antioxidant functions of zinc; however, the mechanisms by which zinc protects macromolecules from oxidative modification are not completely understood. The current study shows that in the circulation, plasma antioxidant scavenger concentrations (FRAP, ascorbic acid, and α -tocopherol) and erythrocyte SOD activity are not directly affected by dietary zinc. However, unlike plasma α -tocopherol, hepatic α -tocopherol levels were reduced with zinc depletion. On the other hand, plasma F₂-isoprostanes levels, an index of lipid peroxidation, were the same in the ZD and PF rats. It is possible that the vitamin E status in the liver was compromised to maintain plasma α -tocopherol levels and thus suppresses lipid peroxidation caused by zinc depletion. However, further studies are required to confirm the effects of zinc deficiency on the metabolism and transportation of hepatic vitamin E.

Although zinc may not regulate antioxidant defenses directly, several other mechanisms could be involved in the antioxidant activity of zinc. First, zinc protects sulfhydryl groups in proteins from oxidation and helps maintain normal functions of proteins (29). Zinc may modulate the oxido-reductive environment in cells through modulation of thiol status. Thus, zinc depletion *in vivo* may change the intracellular environment from a reductive to a more oxidative state (13,30–32) and make cells vulnerable to oxidative stress. Second, zinc antagonizes the activities of bivalent transition metals, including iron and copper, and

TABLE 1 Plasma and tissue antioxidant status in rats fed a ZD, PF, or ZA diet for 21 d and in ZnRe rats fed a ZD diet for 21 d and then a ZA diet for 10 d¹

Group	Plasma	Plasma	Plasma	Hepatic	Erythrocyte
	FRAP	ascorbic acid	α -tocopherol	α -tocopherol	SOD activity
		$\mu\text{mol/L}$		nmol/g	U/mg Hb^2
ZA	247.0 \pm 21.8 ^a	79.3 \pm 7.8 ^a	18.8 \pm 1.0 ^a	127.1 \pm 12.2 ^a	100.1 \pm 4.2
ZD	169.2 \pm 5.8 ^b	31.4 \pm 2.8 ^b	13.0 \pm 0.9 ^b	58.1 \pm 6.9 ^c	101.7 \pm 4.1
PF	160.9 \pm 5.1 ^b	28.3 \pm 3.7 ^b	14.0 \pm 0.9 ^b	90.8 \pm 9.8 ^b	105.1 \pm 4.2
ZnRe	258.9 \pm 9.6 ^a	75.0 \pm 7.0 ^a	20.7 \pm 0.7 ^a	100.7 \pm 5.6 ^{a,b}	89.0 \pm 6.6
<i>P</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	0.12

¹ Values are means \pm SEM, $n = 10$. Means in a column with superscripts without a common letter differ, $P < 0.05$.

² Hb, Hemoglobin.

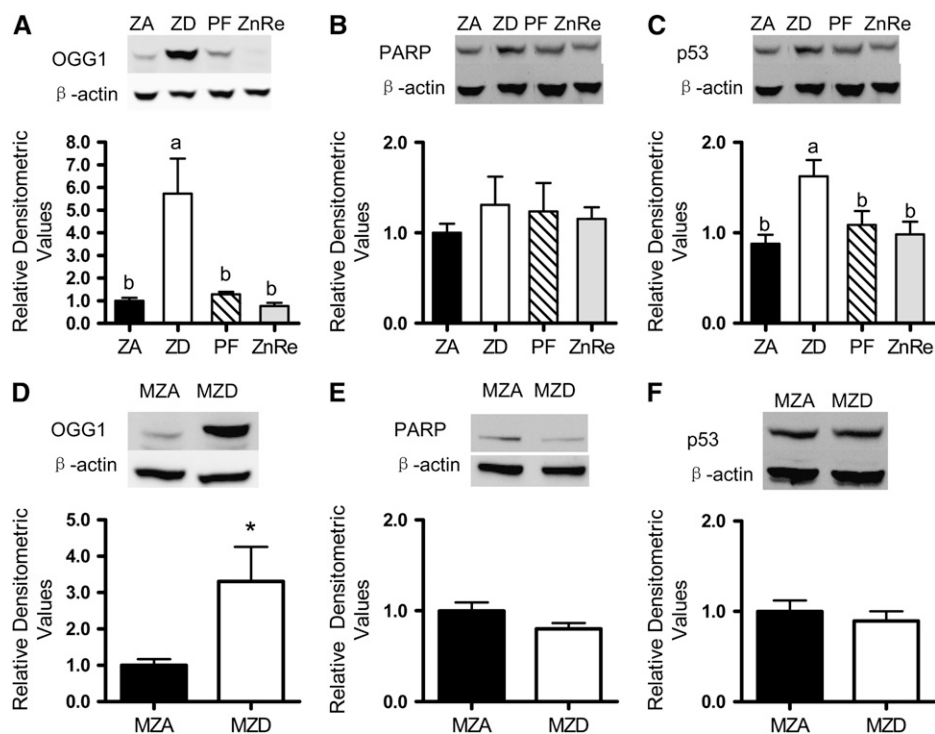


FIGURE 4 Effects of dietary zinc status on the protein levels of DNA repair proteins OGG1(A,D), PARP(B,E), and p53(C,F) in rat livers. Representative western blots are shown in the inserts. Values are means \pm SEM, $n = 6$. (A–C) Means without a common letter differ, $P < 0.05$. (D–F) *Different from the MZA, $P < 0.05$.

prevents the deleterious free-radical reactions (e.g. Fenton reaction) stimulated by iron and copper. Third, zinc is a component of metallothioneins that are part of classic antioxidant defenses. Metallothionein levels are decreased by zinc deficiency in liver and esophagus (33,34). Our laboratory has also detected a decrease in prostate metallothionein expression with zinc deficiency in rats (M. Yan and E. Ho, unpublished data). A lack of metallothionein may further sensitize cells to oxidative insults. Further exploration of these potential mechanisms is an important area of future research.

Increases in DNA damage with zinc deficiency may not be due only to perturbations in oxidative stress but also to compromised DNA repair functions. In the current study, repletion of zinc deficiency reversed DNA damage in peripheral blood cells containing both short-lived neutrophils and long-lived lymphocytes, suggesting that DNA damage is repaired in long-lived cells and/or eliminated with damaged cells through cell death or cell apoptosis. Normally, accumulation of DNA damage stimulates cell responses, including DNA repair, cell cycle arrest, and apoptosis, which help repair DNA damage and inhibit the accumulation of mutations. However, in zinc-deficient rats, DNA damage responses may be compromised, which causes the accumulation of DNA damage and possible increases in cancer risk.

Different mechanisms could be involved in altering DNA damage responses in zinc-deficient rats. Loss of intracellular zinc may decrease the expression or impair the function of DNA repair proteins, thereby interrupting DNA repair pathways. For example, p53 is a zinc-containing protein that plays an essential role in regulating DNA repair, cell proliferation, and cell death (35). Zinc is located in the DNA binding domain and is essential for the DNA binding activity of p53. p53 expression is induced in zinc-depleted cells and rats (5,35,36). However, the current study and our previous *in vitro* studies (28,36) show that the DNA binding activity of p53 is impaired by zinc deficiency. The current study also assessed 2 other DNA repair proteins, PARP and OGG1, which play pivotal roles in the base excision repair

(BER) pathway. 8-Hydroxyl-2'-deoxyguanosine is a biomarker for oxidative DNA damage and is one of the major targets of BER. OGG1 functions in the first step of the BER pathway to recognize and remove 8-hydroxyl-2'-deoxyguanosine (37). PARP binds to DNA SSB created by OGG1 through its zinc finger motif and recruits other DNA repair factors (e.g. X-ray repair cross complementing gene and DNA ligase) to the nick to complete the whole repair process (38,39). Although both of them have zinc-finger motifs, zinc status appears to affect their expression differently. These results point out a potential hierarchy of zinc-related protein function that may be preserved in the absence of cellular zinc levels. This study shows that OGG1 expression is dramatically increased by marginal and severe zinc depletion, indicating increased oxidative DNA damage with zinc deficiency. However, PARP capacity and expression is decreased or only slightly altered with zinc deficiency (40,41). This lack of response or negative response of PARP to zinc depletion could markedly interrupt the overall BER pathway and contribute to the accumulation of DNA damage. Although marginal and severe zinc deficiency may affect DNA repair proteins differently, overall, they both impair DNA repair functions and disable cells to get rid of oxidative DNA damage.

In the United States, ~12% people do not consume enough zinc (2). Infants, children, women, and elderly people are at high risk of marginal zinc deficiency because of either high nutrient requirements or compromised digestion and absorption functions (42). This study confirms that zinc depletion, including marginal zinc deficiency, promotes DNA damage. The impairment of DNA integrity could have an important impact on several processes involved in immune function, cancer, and other degenerative disorders. However, these deleterious effects of zinc deficiency on DNA integrity do appear to be reversible. The results of this study suggest that there are complex *in vivo* interactions among zinc deficiency, DNA integrity, oxidative stress, and DNA repair and suggest a role for zinc in maintaining DNA integrity.

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