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Dietary Zinc Regulates Apoptosis through the Phosphorylated Eukaryotic Initiation Factor 2α/Activating Transcription Factor-4/C/EBP-Homologous Protein Pathway during Pharmacologically Induced Endoplasmic Reticulum Stress in Livers of Mice[1–3]

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Abstract

Background: Several in vitro studies have shown that zinc deficiency could induce endoplasmic reticulum (ER) stress, resulting in activation of the unfolded protein response.

Objective: We aimed to determine whether consumption of a zinc-deficient diet (ZnD) triggers ER stress and to understand the impact of dietary zinc intake on ER stress–induced apoptosis using a mouse model.

Methods: Young adult (8–16 wk of age) male mice of strain C57BL/6 were fed either a ZnD (<1 mg/kg diet), or a zinc-adequate diet (ZnA; 30 mg/kg diet). After 2 wk, liver, pancreas, and serum samples were collected and analyzed for indexes of ER stress. In another experiment, mice were fed either a ZnD, a ZnA, or a zinc-supplementation diet (ZnS; 180 mg/kg diet). After 2 wk, vehicle or tunicamycin (TM; 2 mg/kg body weight) was administered to mice to model ER stress. Liver and serum were analyzed for indexes of ER stress to evaluate the effects of zinc status.

Results: Mice fed a ZnD did not activate the apoptotic and ER stress markers in the liver or pancreas. During the TM challenge, mice fed a ZnD showed greater C/EBP-homologous protein expression in the liver (3.8-fold, $P < 0.01$) than did ZnA-fed mice. TM-treated mice fed a ZnD also had greater terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling–positive cells in the liver (2.2-fold, $P < 0.05$), greater hepatic triglyceride accumulation (1.5-fold, $P < 0.05$), greater serum alanine aminotransferase activity (1.6-fold, $P < 0.05$), and greater protein-tyrosine phosphatase 1B activity (1.5-fold, $P < 0.05$), respectively, than did those fed a ZnA. No significant differences were observed in these parameters between mice fed ZnAs and ZnSs.

Conclusions: Consumption of a ZnD per se is not a critical factor for induction of ER stress in mice; however, once ER stress is triggered, adequate dietary zinc intake is required for suppressing apoptotic cell death and further insults in the liver of mice. *J Nutr* 2016;146:2180–6.

Keywords: zinc-deficient diet, endoplasmic reticulum stress, unfolded protein response, apoptosis, protein tyrosine phosphatase 1B

Introduction

The endoplasmic reticulum (ER)[4] is a cellular organelle responsible for the folding, assembly, and trafficking of proteins. Only appropriately folded proteins are transferred to the Golgi apparatus for distribution to their final locations (1). Incorrectly folded proteins are retained in the lumen of the ER and are

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[3] Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[4] Abbreviations used: ALT, alanine aminotransferase; ATF, activating transcription factor; CHOP, C/EBP-homologous protein; ER, endoplasmic reticulum; GRP78, 78-kDa glucose-regulated protein; GRP94, 94-kDa glucose-regulated protein; p-eIF2α, phosphorylated eukaryotic initiation factor 2α; PTP1B, protein-tyrosine phosphatase 1B; TM, tunicamycin; TPEN, N,N,N′,N′-tetrakis (2-pyridylmethyl)ethylenediamine; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling; UPR, unfolded protein response; ZIP, zrt/irt-like protein; ZnA, zinc-adequate diet; ZnD, zinc-deficient diet; ZnS, zinc-supplementation diet.

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diverted to the cytosol to be degraded by proteasomes through ER-associated degradation (2). When the ER function is compromised, however, misfolded proteins accumulate in the ER, which leads to ER stress (3). A variety of physiological insults, including viral infection, oxidative stress, long-term consumption of a high-fat diet, hypoxia, chronic alcohol consumption, and glucose deprivation, have been identified to impair ER-folding capacities, resulting in ER stress (4–7).

Because prolonged ER stress is toxic to cells, eukaryotic cells activate a special pathway called the unfolded protein response (UPR) to alleviate ER stress (8). UPR restores ER homeostasis by reducing the translation of mRNA through the phosphorylation of eukaryotic initiation factor 2α (p-eIF2α), by enhancing protein-degrading abilities through activation of X-box binding protein-1 (XBP-1) and activating transcription factor (ATF)-6 (8). ATF6 activation also leads to increased expression of ER chaperones such as 78-kDa glucose-regulated protein (GRP78/binding immunoglobulin protein) and 94-kDa glucose-regulated protein (GRP94) to assist in protein folding (9). If ER stress is too severe to be overcome, however, then UPR leads cells to apoptotic cell death via phosphorylated eIF2α-mediated expression of ATF4 and its downstream effector, C/EBP-homologous protein (CHOP) (10, 11). Therefore, suppression of the proapoptotic p-eIF2α/ATF4/CHOP pathway is a key mechanism in the adaptation and overcoming of ER stress. Extensive ER stress–induced apoptosis has been linked to many pathogenic disorders such as neurodegenerative diseases, diabetes, cancer, and obesity (12). In the liver, ER stress–mediated apoptosis can induce hepatic steatosis as a result of an aberrant regulation of multiple metabolic transcription factors that regulate lipid homeostasis (13, 14).

Zinc is an essential trace mineral that plays important roles as a structural or a catalytic cofactor (15). It also can regulate gene expression and signal transduction, mediating cellular metabolism (16). Although zinc is indispensable to cells, a high intracellular labile zinc concentration is potentially toxic. Thus, zinc distribution to subcellular compartments such as mitochondria, Golgi apparatus, and vesicles is tightly regulated (17). Control of zinc distribution provides zinc to each organelle to maintain zinc-dependent functions (18). The ER also serves as the intracellular zinc distribution site, where newly synthesized proteins can acquire zinc during the folding processes and in the secretory pathway (19, 20).

Systemic zinc deficiency has been associated with a number of human disorders such as growth retardation, immunodeficiency, dermatitis, hypogonadism, and neurodegenerative disorders (15, 21, 22). In addition, zinc deficiency has been implicated in ER stress. Zinc restriction mediated by treating cells with the cell-permeable zinc chelator *N,N,N′,N′*-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN), induces ER stress and results in the activation of UPR in some mammalian cell lines (23). In HeLa cells, zinc deficiency in cytosol and other organelles induced by knockdown of the *ZIP13* (zrt/irt-like protein) gene (*SLC39A13*), which transports vesicular zinc to cytosol, has been shown to cause ER stress (19). It also has been demonstrated with an animal model that hepatic zinc deficiency mediated by chronic ethanol exposure in rats triggered ER stress–mediated apoptotic cell death (24).

To our knowledge, it has not been established whether consumption of a zinc-deficient diet (ZnD) can induce ER stress using an in vivo murine model. Furthermore, the impact of dietary zinc intake on multiple indexes of ER stress is unclear. To obtain answers to these questions, we fed mice with either a ZnD, a zinc-adequate diet (ZnA), or a zinc-supplementation diet (ZnS) for 2 wk; then tunicamycin (TM), a potent ER stress inducer, was administered to model ER stress. In mice, the consumption of a ZnD alone did not activate the UPR; however, dietary zinc content was a critical factor in their adaptation to pharmacologically induced ER stress via suppressing apoptosis in liver.

Methods

Animals, diets, and treatments. Young adult (8–16 wk of age) male mice of strain C57BL/6 were used throughout this study. All purified diets were purchased from Research Diets. Mice were fed egg white–based purified diets (AIN-76A) that contained 30 mg Zn/kg for a 5-d acclimation period (25). For 2 wk thereafter the mice were fed the same diet formulation, which contained <1 mg Zn/kg (ZnD), 30 mg Zn/kg (ZnA), or 180 mg Zn/kg (ZnS). The mice had free access to food and Milli-Q drinking water (Millipore Corporation) until killed. Each mouse was individually housed using stainless steel hanging wire cages under husbandry conditions described elsewhere (26, 27). In experiments modeling ER stress, TM (Sigma) dissolved in 1% DMSO · 150 $mmol^{-1} \cdot L$ $glucose^{-1}$ was administered to mice at 2 mg/kg body weight via a single intraperitoneal injection. The timing of the injection was determined by time of killing. Mice were anesthetized by isofluorane inhalation before injections and were killed by cardiac puncture. Mice were killed between 0900 and 1000. Tissues were excised following laparotomy, snap frozen in liquid nitrogen, and stored at −80°C. All of the research protocols were approved by the University of Florida Institutional Animal Care and Use Committee.

RNA isolation and real-time qPCR. Total RNA from collected tissues was isolated by using TRIzol reagent (Ambion) and homogenized using a Bullet Blender (Next Advance). To prevent DNA contamination, isolated total RNA was treated by Turbo DNA-free reagent (Ambion). qPCR was performed using EXPRESS One-Step Superscript Mix (Invitrogen) and StepOnePlus Fast Thermocycler (Applied Biosystems). Amplification values were normalized to TATA-binding protein mRNA (28). The following are the primer and probe sequences used: GRP78: forward: 5′-TTCTGCCATGGTTCTCACTAAA-3′, reverse: 5′-TTGTCGCTGGGCATCATT-3′, probe: 5′-FAM-AGACTGCTGAGGCGTATTTGGGAA-BHQ1–3′; CHOP: forward: 5′-CAGCGACAGAGCCAGAATAA-3′, reverse: 5′-CAGGTGTGGTGGTGTATGAA-3′, probe: 5′-FAM-TGAGGAGAGAGTGTTCCAGAAGGAAGT-BHQ1–3′; TATA-binding protein: forward: 5′-TCTGCGGTCGCGTCATT-3′, reverse: 5′-GGGTTATCTTCACACACCATGAAA-3′, probe: 5′-FAM-TCTCCGCAGTGCCCAGCATCA-BHQ1–3′.

Western blotting. Tissues were homogenized in RIPA lysis buffer (Santa Cruz) supplemented with protease and phosphatase inhibitors (Thermo Fisher Scientific). Proteins were separated by SDS-PAGE (10% acrylamide gel), transferred to nitrocellulose membranes, and probed with primary antibodies (27, 28). The antibodies purchased were GRP78, CHOP, p-eIF2α (Ser^{52}) (Santa Cruz Biotechnology), ATF4, GRP94 (Cell Signaling), and tubulin (Abcam). Primary antibodies were detected by horseradish peroxidase–conjugated anti-rabbit immunoglobulin G (GE Healthcare). Immunoreactivity was visualized with chemiluminescence reagents (Thermo Fisher Scientific).

Biochemical analyses. The serum zinc concentration was measured using flame atomic absorption spectrophotometry. Blood was collected via cardiac puncture, and serum was obtained by centrifugation and was diluted 1:3 using Milli-Q water for analysis. For determination of tissue zinc concentrations, weighed tissue samples were digested in HNO_3 at 90°C for 3 h, and samples were diluted 1:2 with Milli-Q water. After flame atomic absorption spectrophotometry analysis, total zinc values were normalized to wet weight of tissue. Serum alanine aminotransferase (ALT) activity was quantified by a colorimetric endpoint method, as previously described (28). Live TGs were measured using reagents provided by BioVision Research Products, following their instructions.

Histological analysis. Liver tissues were fixed in 10% formalin in PBS, embedded in paraffin, and sectioned to a thickness of 4 μm . The paraffin sections were stained with hematoxylin and eosin. Apoptotic cells in the liver were detected by terminal deoxynucleotidyltransferase–mediated

deoxyuridine triphosphate nick-end labeling (TUNEL) assay by use of an in situ apoptosis detection assay (Abcam), according to the manufacturer's instructions. The lipid droplet areas were determined using ImageJ software (National Institute of Mental Health).

Protein-tyrosine phosphatase 1B assay. Protein-tyrosine phosphatase 1B (PTP1B) activity was measured as described previously (28), with slight modifications. Briefly, total lysates were obtained by homogenization of liver tissue using a HEPES buffer supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific) and the Bullet Blender (Next Advance). After centrifugation at 13,000 × *g* at 4°C, the supernatant was incubated with PTP1B substrate (ELEF-pY-MDYE-NH2) (AnaSpec) for 30 min at 30°C. Sodium orthovanadate, a nonspecific phosphatase inhibitor (Sigma), and 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-*N*-{4-[(2-thiazolylamino)sulfonyl] phenyl}-6-benzofuransulfonamide, a PTP1B-specific inhibitor (Calbiochem), were used as positive controls of phosphatase activity inhibition. Released inorganic phosphate was measured using a colorimetric phosphate assay (Biovision). Assays were normalized to total protein concentration (BioRad).

Statistical analyses. All of the statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software). Results are expressed as means ± SDs. Comparisons between 2 groups were analyzed by Student's *t* test, and comparison between multiple groups were analyzed using 1- or 2-factor ANOVA followed by a Tukey's test. The statistical significance was set at $P < 0.05$.

Results

No activation of ER stress markers in mice fed a ZnD. The husbandry methods used did not influence food intake or body weight during the 2-wk comparison period. We aimed to examine whether consumption of a ZnD induces ER stress in a mouse model. After 2 wk of the controlled zinc intake period, mice fed a ZnD displayed significantly decreased serum, liver, and pancreatic zinc concentrations compared with those of mice fed a ZnA, demonstrating that a ZnD triggered systemic zinc depletion in mice (**Figure 1**A) . Of note is the drastic reduction in metallothionein-1 mRNA levels in both liver and pancreas (Figures 1B and 1D). These results demonstrate that zinc restriction produced by feeding the ZnD was effective in decreasing intracellular zinc pools. Because liver and pancreas are major organs that are affected greatly by ER stress, gene and protein expression of GRP78 and CHOP, which are common markers of UPR activation (29), were analyzed in these 2 organs. No difference in expression of GRP78 and CHOP mRNA was observed in the tissues of mice fed a ZnD, however (Figures 1C and 1E). CHOP protein expression was not detected by immunoblot in either tissue. GPR78 was not different as shown by densitometry of individual Western blots (Figures 1F and 1G). These results indicate that diet-mediated zinc deficiency is not a factor that causes ER stress in a mouse model with documented signs of intracellular zinc deprivation.

Although zinc deficiency mediated by the ZnD did not cause ER stress in mice, we hypothesized that zinc may play a role during induced ER stress. As such, our next step was to investigate the response of the liver to pharmacologically induced ER stress when the zinc content of the diet was varied. After 2 wk, the concentrations of serum zinc (data not shown), and liver

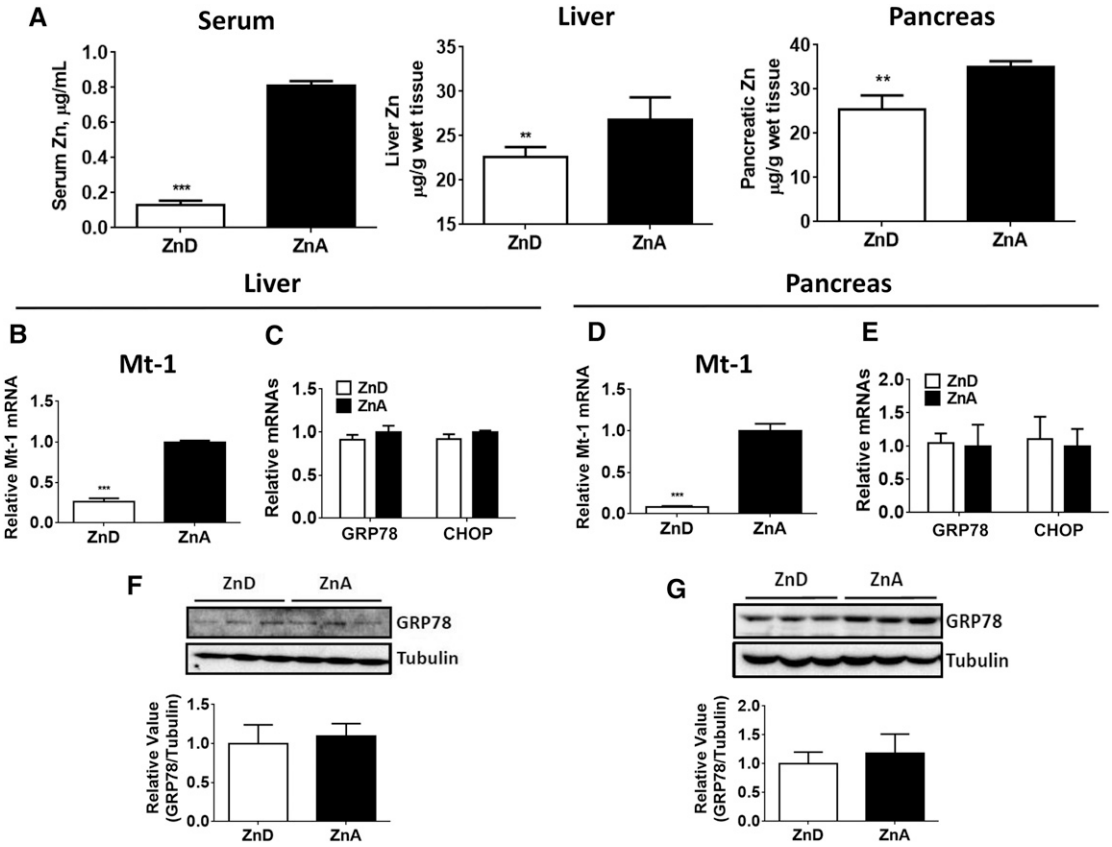

FIGURE 1 Serum, liver, and pancreatic zinc concentration (A), quantitative PCR of mRNA of Mt-1, GRP78, and CHOP (B–E), and Western blot analysis of GRP78 (F, G) in liver (B, C, F) and pancreas (D, E, G) of mice fed ZnDs or ZnAs for 2 wk. In panels F and G, individual blots were analyzed by digital densitometry. Values are means ± SDs (n = 3–4 mice/group). $^{**}P < 0.01$, $^{***}P < 0.001$ (unpaired Student's *t* test). CHOP, C/EBP-homologous protein; GRP78, 78-kDa glucose-regulated protein; Mt-1, metallothionein-1, ZnA, zinc-adequate diet; ZnD, zinc-deficient diet.

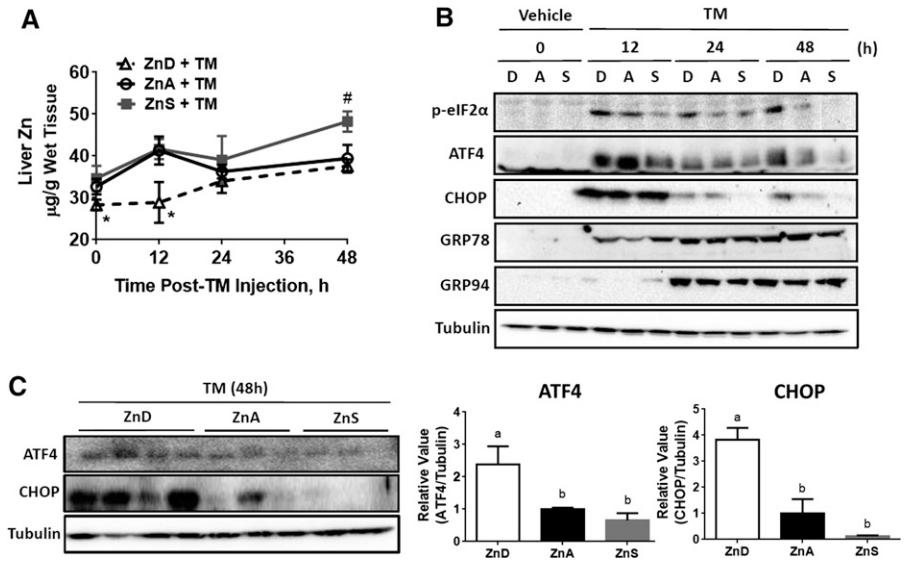

FIGURE 2 Zinc concentration (A) and Western blot analysis of markers of ER stress (B, C) in livers of mice injected with TM (2 mg/kg) or vehicle for the indicated time following ZnD, ZnA, or ZnS for 2 wk. In panel A, values are means ± SDs (n = 3–4 mice/group). $^{*}P < 0.05$ compared with ZnA or ZnS, $^{\#}P < 0.05$ compared with ZnD or ZnA (2-factor ANOVA followed by Tukey's multiple comparison tests at each time point). In panel B, pooled samples were blotted (n = 3–4 pooled samples/group). In panel C, individual samples were blotted and analyzed by digital densitometry. Values are means ± SDs (n = 3–4 mice/group). Labeled means without a common letter differ; $P < 0.05$ (1-factor ANOVA followed by Tukey's multiple comparison tests). A and ZnA, zinc-adequate diet; ATF4, activating transcription factor-4; CHOP, C/EBP-homologous protein; D and ZnD, zinc-deficient diet; ER, endoplasmic reticulum; GRP78, 78 kDa glucose-regulated protein; GRP94, 94-kDa glucose-regulated protein; p-eIF2α, phosphorylated eukaryote-initiation factor 2α; S and ZnS, zinc-supplementation diet; TM, tunicamycin.

zinc (**Figure 2**A, see 0 h) reflected the amount of dietary zinc provided to each group. Following the TM injection (2 mg/kg), extra zinc accumulated in the liver during ER stress (Figure 2A). The ZnD group had a substantially lower ($P < 0.05$) amount of extrahepatic zinc accumulation by 12 h after TM administration. In contrast, the ZnS group had a substantially greater ($P < 0.05$) amount of extrahepatic zinc accumulation by 48 h after the TM was administered.

Dietary zinc is essential for suppression of ER stress-induced apoptosis. To examine whether the dietary zinc content influences the adaptation to ER stress, markers of UPR were analyzed. In comparison to the ZnA, mice fed a ZnD expressed greater concentrations of p-eIF2α, ATF4, and CHOP ($P < 0.05$), especially later in the TM challenge (e.g., 24 and 48 h) (Figures 2B and C). On the contrary, mice fed a ZnS demonstrated less expression of those proteins. These differences

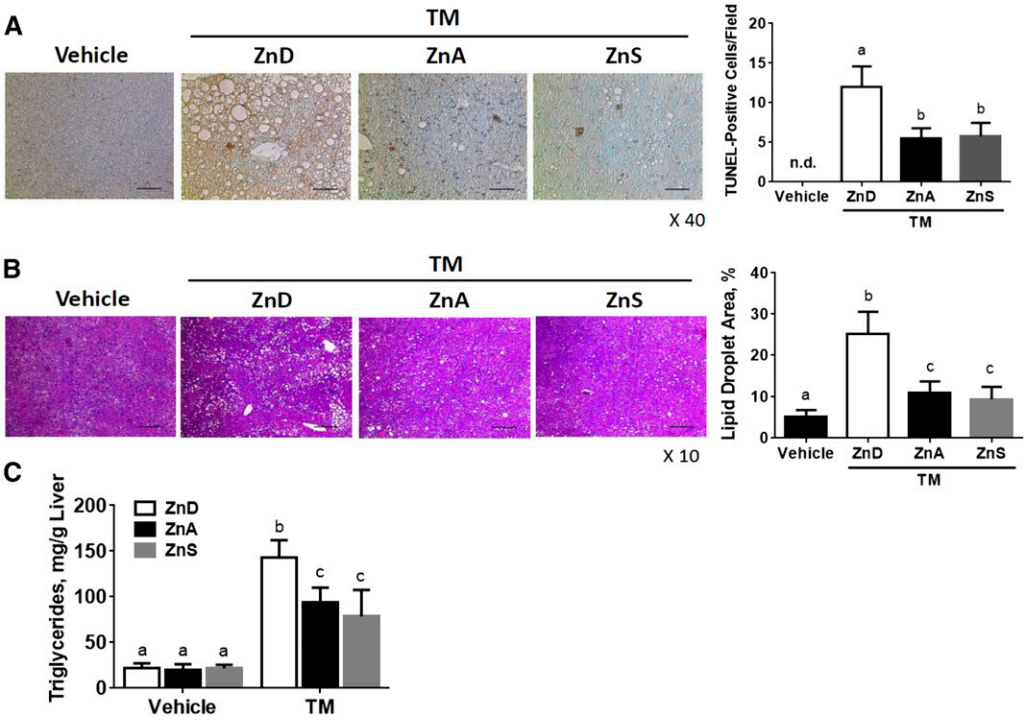

FIGURE 3 Representative images of TUNEL assay (A) and H&E staining (B) and quantification of TGs (C) in livers of mice injected with TM (2 mg/kg) or vehicle for 48 h following ZnD, ZnA, or ZnS for 2 wk. Values are means ± SDs (n = 3–4 mice/group). Labeled means without a common letter differ; $P < 0.05$ (2-factor ANOVA followed by Tukey's multiple comparison tests). In panel A, representative images are at 40× magnification; bars = 25 μm. In panel B, representative images are at 10× magnification; bars = 100 μm. H&E, hematoxylin and eosin; n.d., not detected; TM, tunicamycin; TUNEL, terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling; ZnA, zinc-adequate diet; ZnD, zinc-deficient diet; ZnS, zinc-supplementation diet.

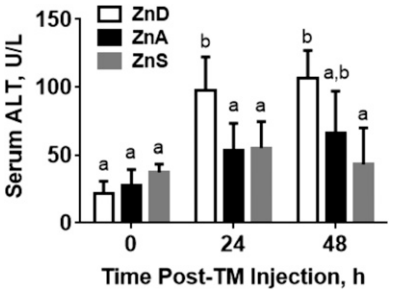

FIGURE 4 Serum ALT activity was measured in livers of mice injected with TM (2 mg/kg) for the indicated time following ZnD, ZnA, or ZnS for 2 wk. Values are means ± SDs (n = 3–4 mice/group). Labeled means without a common letter differ; $P < 0.05$ (2-factor ANOVA followed by Tukey's multiple comparison tests). ALT, alanine aminotransferase; TM, tunicamycin; ZnA, zinc-adequate diet; ZnD, zinc-deficient diet; ZnS, zinc-supplementation diet.

between the ZnA and ZnS groups were not significantly different, however (Figure 2C). Expression of GRP78 and GRP94 tended to increase in the ZnD group, suggesting that requirement for ER chaperones might be enhanced in ZnD group to help protein folding (Figure 2B). To confirm the western blot data, the level of apoptosis was evaluated by using TUNEL assay in sectioned liver tissue (**Figure 3**A). Consistent with proapoptotic protein expression patterns, significantly ($P < 0.05$) greater concentrations of TUNEL-positive cells (dark brown) were observed in the livers of mice fed a ZnD during the TM challenge compared with those fed ZnAs and ZnSs. There was no difference in TUNEL-positive cells between ZnAs and ZnSs (Figure 3A). These observations suggest that adequate dietary zinc is required for suppression of ER stress–mediated apoptotic cell death via modulation of the proapoptotic p-eIF2α/ATF4/CHOP pathway.

Hepatic steatosis and tissue damage has been shown to be caused by ER stress (13, 14). To test for the accumulation of lipid droplets in our mice, liver sections were stained with hematoxylin and eosin. As shown in Figure 3B, a significantly ($P < 0.05$) greater amount of lipid droplet accumulation (white dots) was detected in mice fed a ZnD in response to TM than in those fed ZnAs and ZnSs. No difference was observed between ZnAs and ZnSs. The livers of TM-treated mice had a greater accumulation of TGs ($P < 0.001$) than did vehicle-treated mice (Figure 3C). During the TM challenge, the TG content of the liver of mice fed the ZnD was greater ($P < 0.05$) than that of mice fed the ZnAs or ZnSs.

Serum ALT was measured to analyze the liver tissue damage caused by ER stress (**Figure 4**). Serum ALT concentration is a common marker of liver damage because the ALT that is present in hepatocytes is leaked to plasma by damaged cells (30). Serum of mice fed a ZnD displayed greater amounts ($P < 0.05$) of ALT activity during TM treatment than did mice fed ZnAs or ZnSs.

During ER stress, PTP1B activity increased in the mice fed a ZnD. PTP1B is a well-known regulator of the insulin signaling pathway and has been implicated in the UPR pathway (31). It has been shown that ER stress increases hepatic PTP1B expression, and liver-specific knockdown of PTP1B in mice produced an apoptosis-resistant phenotype during ER stress (31). Interestingly, zinc is a known inhibitor of PTP1B in cells (32), which led us to consider this enzyme to be a potential mediator that associates zinc and ER stress–induced apoptosis. During the TM challenge, PTP1B activity was significantly elevated ($P < 0.05$) in mice fed a ZnD, whereas the activities in the mice fed ZnAs and ZnSs were not different from vehicle-treated controls (**Figure 5**B). These results suggest that the higher levels of ER stress–induced apoptosis in the mice fed a ZnD may result from the elevated PTP1B activity as a result of the low availability of zinc.

Discussion

Zinc is essential for normal ER function, and its deficiency has been suggested as a cause of ER stress (20, 23). The perception is based largely on in vitro experiments that used TPEN, a cell-permeable zinc chelator, to restrict available zinc (23, 33). Indeed, in previous reports, the addition of TPEN to cells activated the UPR pathway in mammalian cells. These conditions produce an unphysiologic equimolar chelation of TPEN: Zn that leads to cell death (34–36). To investigate zinc depletion at the in vivo level, we fed mice a ZnD for 2 wk and analyzed markers of both zinc status and ER stress. After 2 wk, however, there was no activation of UPR markers in mice fed the ZnD that yielded signs of dietary zinc deficiency. The discrepancy between previous in vitro experiments and our in vivo experiments may be derived in part from the systemic homeostatic mechanisms in animals. Human and animals fed a low-zinc diet can react to a reduced zinc source to maintain cellular zinc homeostasis (15). Our data shows a statistically significant but mild decrease in the hepatic zinc concentration of mice fed a ZnD compared with that of a ZnA. Consumption of a low-zinc diet may not, therefore, be a critical factor that triggers ER stress in vivo; however, we are aware of the possibility that

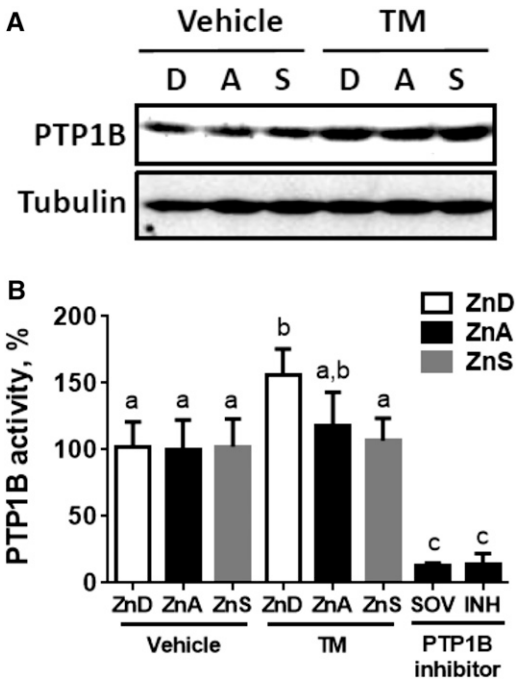

FIGURE 5 Western blot analysis (A) and activity assay (B) of PTP1B in liver of mice injected with TM (2 mg/kg) for 48 h following ZnD, ZnA, or ZnS for 2 wk. In panel A, pooled samples from 3–4 mice/group were used. In panel B, individual samples from 3–4 mice/group were assayed. Values are means ± SDs (n = 3–4 mice/group). Labeled means without a common letter differ; $P < 0.05$ (2-factor ANOVA followed by Tukey's multiple comparison tests). A and ZnA, zinc-adequate diet; D and ZnD, zinc-deficient diet; INH (PTP1B-specific inhibitor), 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-*N*-[4-[(2-thiazolylamino) sulfonyl]phenyl]-6-benzofuransulfonamide; PTP1B, protein-tyrosine phosphatase 1B; S and ZnS, zinc-supplementation diet; SOV (non-specific phosphatase inhibitor), sodium orthovanadate; TM, tunicamycin.

zinc deficiency may cause ER stress in a much longer and more severe zinc-deficiency setting. We used a 2-wk-long period of dietary zinc manipulation, which was enough time to induce systemic zinc deficiency in the mouse model according to our previous observations (27, 35). We tried to avoid long-term (e.g., >1 mo) ZnD feeding to mice because it could trigger a number of complications and is not a likely representation of zinc deficiency in humans.

A number of pathologic conditions, such as diabetes, neurological disorders, and hepatic steatosis, are related to ER stress (12). Disturbed zinc homeostasis has been implicated in those diseases (25, 37–39). As such, we aimed to investigate the impact of dietary zinc status on induced ER stress using TM administration to mice. When intraperitoneally injected into mice, TM has been reported to affect mainly the liver and kidney (40), where TM leads to apoptotic cell death or further insults, especially when the key component of UPR is impaired (41, 42). In this study, we focused on the liver in particular because it is the site where a massive number of proteins are synthesized, which makes this organ more susceptible to perturbation of protein folding. As shown in Figure 2A, extra zinc accumulated in liver during the TM challenge, which suggests that the requirement of zinc may be enhanced in this organ during ER stress. The event may be mediated by elevated expression of zinc transporters, including ZIP14, which locates on the cell membrane and transports extracellular zinc into cells. ZIP14 expression is abundant in the liver. Homma et al. reported increased ZIP14 mRNA expression in response to TM in mice liver (23). We also have found that expression of ZIP14 increases in the same setting (M-H Kim, TB Aydemir, RJ Cousins, unpublished data).

There are potential reasons for extrahepatic zinc accumulation during ER stress. First, more zinc may be required to help the protein folding process under this stress condition as a structural component of many proteins or as a regulatory factor (15). For example, zinc is a cofactor of calreticulin, which is an ER resident protein that prevents transport of misfolded proteins from the ER to the Golgi apparatus (43, 44). Second, our data support the notion that more zinc may be required to suppress ER stress–induced apoptosis. Zinc deficiency has been implicated in apoptosis in both in vitro and in vivo models (45–47). UPR comprises the prosurvival pathway and the proapoptosis pathway, and both pathways are activated with ER stress. To adapt and survive against ER stress, the proapoptotic p-eIF2α/ATF4/CHOP pathway should be suppressed (48). In particular, the induction of CHOP has been shown to be crucial for ER stress–induced apoptosis, suggesting that CHOP can be a target protein leading to adaptation (49, 50). We suggest that dietary zinc is associated with this pathway. In response to the TM challenge, mice fed a ZnD had higher concentrations of the proapoptotic protein expressions and TUNEL-positive cells than did mice fed ZnAs and ZnSs. If ER stress is not resolved through the activation of UPR, cells undergo chronic ER stress characterized by prolonged apoptotic cell death, which accompanies a variety of pathologic outcomes (11, 12). For instance, chronic ER stress in the liver results in lipid droplet accumulation (hepatic steatosis) (14). Excessive lipid accumulation in response to TM challenge was observed in mice fed the ZnD (Figure 3), and they showed higher amounts of liver damage (Figure 4). In agreement with our results, Sun et al. (24) reported that zinc deficiency induces ER stress–mediated apoptosis in the livers of rats fed ethanol long term. Collectively, our findings suggest that a diet providing an adequate source of zinc is critical in adaptation to ER stress by suppressing apoptotic cell death.

PTP1B is a member of the protein tyrosine phosphatase family, which can regulate a number of pathways (51, 52). One well-known example is the insulin signaling pathway, in which PTP1B acts as a negative regulator (53). In addition, PTP1B has been implicated in ER stress. In response to ER stress, PTP1B expression was enhanced in vitro and in vivo; liver-specific PTP1B knockout mice could attenuate ER stress and had decreased indexes of metabolic syndrome (32, 54). Ablation of PTP1B has been shown to lower expression of UPR markers, including ATF4 and CHOP, during TM administration and 16 wk of high-fat diet feeding (53). We suggest that the antiapoptotic effect of zinc during ER stress may be mediated by PTP1B because zinc can inhibit PTP1B activity through binding (33). After TM treatment, we found elevated levels of hepatic PTP1B activity in mice fed a ZnD, which may have resulted from the low availability of zinc (Figure 5B). In contrast, the PTP1B activity of mice fed ZnAs and ZnSs did not increase during the TM challenge, which may be the result of extra zinc uptake, and hence low activity, in the liver compared with the mice fed a ZnD. Further investigation would be needed, however, to elucidate whether suppression of ER stress–induced apoptosis by zinc actually is mediated by PTP1B.

In conclusion, we demonstrated that adequate dietary zinc intake was required to adapt to pharmacologically induced ER stress in mouse liver, although consumption of a ZnD alone did not induce ER stress. The zinc-mediated ER stress adaptation was mediated via an effective suppression of the proapoptotic pathway of UPR. Mice fed the ZnD showed higher levels of apoptosis and hepatic steatosis, whereas mice fed adequate amounts of zinc did not display the same conditions. In addition, we suggest that inhibition of PTP1B activity may explain the antiapoptotic effect of zinc during ER stress. Our data raise the possibility that consumption of a ZnD is harmful to individuals with a high risk of ER stress.

Acknowledgments

M-HK and RJC designed the research; M-HK and TBA conducted the research; M-HK, TBA, and RJC analyzed the data and wrote the paper; and RJC had responsibility for the final context. All of the authors read and approved the final manuscript.

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