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Zinc and Redox Signaling: Perturbations Associated with Cardiovascular Disease and Diabetes Mellitus

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Abstract

Cellular signal transduction pathways are influenced by the zinc and redox status of the cell. Numerous chronic diseases, including cardiovascular disease (CVD) and diabetes mellitus (DM), have been associated with impaired zinc utilization and increased oxidative stress. In humans, mutations in the *MT-1A* and *ZnT8* genes, both of which are involved in the maintenance of zinc homeostasis, have been linked with DM development. Changes in levels of intracellular free zinc may exacerbate oxidative stress in CVD and DM by impacting glutathione homeostasis, nitric oxide signaling, and nuclear factor-kappa B–dependent cellular processes. Zinc ions have been shown to influence insulin and leptin signaling *via* the phosphoinositide 3′-kinase/Akt pathway, potentially linking an imbalance of zinc at the cellular level to insulin resistance and dyslipidemia. The oxidative modification of cysteine residues in zinc coordination sites in proteins has been implicated in cellular signaling and regulatory pathways. Despite the many interactions between zinc and cellular stress responses, studies investigating the potential therapeutic benefit of zinc supplementation in the prevention and treatment of oxidative stress-related chronic disease in humans are few and inconsistent. Further well-designed randomized controlled trials are needed to determine the effects of zinc supplementation in populations at various stages of CVD and DM progression. *Antioxid. Redox Signal.* 13, 1549–1573.

Introduction

Oxidative stress and perturbations of zinc homeostasis are recognized as important contributors to the pathophysiology of an increasing number of chronic disorders. The apparent interrelationship between zinc and redox effects in a variety of biochemical pathways, combined with the high prevalence of zinc deficiency worldwide (226), has sparked mounting interest in the possible therapeutic benefit of zinc supplementation for human health. This review examines various aspects of zinc biology and its interaction with redox signaling that are of relevance to cardiovascular disease (CVD) and diabetes mellitus (DM), and appraises the effects of nutritional zinc supplementation on CVD and DM outcomes. The exploration of perturbed zinc and redox signaling in each disorder includes a discussion of zinc deficiency and the difficulties associated with the determination of zinc status in humans. The review concludes by suggesting avenues for future research that would advance current knowledge of zinc and redox interactions in CVD and DM, both being diseases of mounting public health importance.

Cellular Zinc Homeostasis

Zinc is necessary for a wide range of physiological processes. In addition to its numerous structural and catalytic functions, zinc is involved in the regulation of an extensive variety of genes, impacting such diverse processes as protein–protein interactions, fatty acid metabolism, apoptosis, and signal transduction (205). As there is no recognized storage site for zinc, cells are dependent on plasma to supply them with a constant supply of zinc to sustain normal function. In humans, homeostatic mechanisms maintain plasma zinc within a concentration range of approximately 10–18 μmol/L. The zinc in plasma is bound principally (up to 70%) to albumin, from which it is easily exchanged. Although at any one time it comprises only a minute fraction ($\sim$0.1%) of the total body zinc, plasma zinc constitutes a highly mobile pool. In addition to the zinc that is moved in and out of the tissues daily, all absorbed zinc ($\sim$50–75 μmol/day) passes through the plasma compartment (108), with the total zinc flux being in the order of 130 times/day (107).

In a typical eukaryotic cell, the total zinc concentration is approximately 200 μM (151), but differs according to cell type.

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Most intracellular zinc is tightly bound in proteins, with only a small proportion, typically found at concentrations in the picomolar to low nanomolar range, being described as readily exchangeable or free zinc (114). On uptake, the binding of zinc to intracellular proteins appears to be rapid; in cell culture systems, exogenously added zinc ions have been shown to enter the cell within minutes, generating a measurable increase in free intracellular zinc of only 70% despite the total zinc uptake being 300% or more (170). The concentration of cellular free zinc, along with the zinc buffering capacity of the cellular components, determines whether zinc is cytoprotective or cytotoxic (133). An intricate arrangement of cellular homeostatic mechanisms has therefore evolved to regulate the intracellular zinc content and its distribution within the mammalian cell. Although much is yet unknown, the maintenance of cellular zinc homeostasis is believed to involve complex interactions between zinc sensors, such as metal responsive element-binding transcription factor-1 (MTF-1), and cell signaling machinery; the transcriptional and/or posttranslational regulation of two recently discovered classes of zinc transporters, the ZnT (SLC30) and Zrt- and Irt-like protein (Zip) (SLC39) transporter families; the trafficking of zinc through the cell by metallothionein (MT); and the synthesis and/or degradation of proteins that bind zinc with high affinity. An evolving understanding of the functions of zinc transporters, in particular, is offering novel insights into the ability of cells to regulate the uptake and availability of zinc. To date, 10 ZnTs and 14 Zips have been identified in mammals. ZnT and Zip proteins appear to have largely opposite functions in zinc transport; ZnT transporters promote the efflux of cytoplasmic zinc from the cell or its sequestration into intracellular organelles [with the possible exception of a ZnT5 variant that has been reported to be bidirectional (207)], while Zip transporters traffic extracellular or organellar zinc into the cytoplasm. ZnT proteins are commonly found associated with endosomes, Golgi, or endoplasmic reticulum, although ZnT1, in addition to having a vesicular localization, is also found at the plasma membrane, where it is the primary transporter responsible for mediating cellular zinc efflux (42). Conversely, most Zip proteins have been observed at the plasma membrane, although the cellular localization of some of them may change according to physiologic conditions (42).

Genes from both zinc transporter families exhibit tissue-specific expression and differential regulation by zinc (122). Changes in expression of ZnTs and Zips in response to zinc supplementation in humans emphasize the involvement of zinc transporters in zinc homeostasis. In ileostomy patients, supplementation with 25 mg Zn/day generated a decrease in expression of *ZnT1* (but not *ZnT5* or *Zip4*) mRNA and ZnT1, ZnT5, and Zip4 proteins in the intestinal mucosa (43). This result was largely commensurate with observations by the same group of reduced ZnT1, ZnT5, and Zip4 mRNA and protein expression in Caco-2 cells after the zinc concentration of the culture medium was increased to 200 μM from 100 μM [a concentration that in the intestinal lumen is physiological (192)]. The subcellular localization and transport functions of ZnT1, ZnT5, and Zip 4 and their responses to zinc supplementation are consistent with a role for these transporters in the regulation of dietary zinc absorption (43). Zinc supplementation in humans, which generally results in a transient increase in plasma zinc concentrations, also has been shown to influence the abundance of zinc transporter mRNAs and proteins in blood cells. In healthy male subjects, levels of *ZnT1* and *Zip3* mRNA expression in whole blood were found to be increased and decreased, respectively, throughout a 10-day period of zinc supplementation (15 mg/day), with a return to control levels observed upon cessation of the supplement (9).

Cell and animal studies have demonstrated that expression of zinc transporters appears to be regulated not only by zinc but also by a variety of other metals, as well as by hormones and cytokines (122), highlighting that zinc transporters are integral to a range of cellular processes. Zinc transporters and other proteins involved in cellular zinc homeostasis collectively ensure that zinc is distributed to zinc-requiring proteins with considerable specificity so as to minimize the potential for protein misfolding or aberrant signaling effects that may result from nonspecific zinc–protein interactions.

Molecular Mechanisms of Zinc–Protein Interactions

Many of the molecular mechanisms underlying the transport of zinc across membranes and the mobility and deployment of zinc within cells are as yet undefined. The two most likely models of zinc transfer are either that zinc is conveyed directly from one protein to another or that zinc dissociates to the solvent before associating with its target protein. It has been argued that the reconstitution of proteins with free zinc, assuming estimates of its low availability are correct, may be too slow to be biologically feasible (84). The existence of ligand exchange mechanisms for the direct transfer of zinc from occupied to unoccupied zinc binding sites therefore may be necessary to circumvent the kinetic limitations of a dissociation/association mechanism (84). With few exceptions, zinc coordination sites in proteins utilize nitrogen, oxygen, and sulfur donors from histidine, glutamate/aspartate, and cysteine ligands, respectively, to bind zinc (210). The zinc–sulfur interaction is recognized as the predominant mode of binding in proteins (132) and the unique properties of both the zinc ion and the thiol group in combination provide an important means by which zinc transfer between proteins *in vivo* may occur.

Zinc coordination to the sulfur of cysteine is found in catalytic, structural, and protein interface binding sites (8, 209). Zinc has a filled d-shell of electrons and is therefore redox-inert, a property that renders it stable despite the constant oxidoreductive flux of the cellular environment (210) and which may account for its widespread use in proteins; whereas disulfide bonds require an oxidative environment for their stability, a zinc-thiolate motif can assist protein folding by forming a stable brace in the reducing environment of the cell (133). The lewis acid properties of zinc (222) allow it to bind tightly to its thiolate electron donors, yet a remarkable feature of the zinc–sulfur coordination environment is that at the same time the sulfur donors allow for fast zinc exchange and ligand-centred redox activity (132). In a phenomenon known as a redox zinc switch, the thiol groups of zinc-coordinating cysteines can be reversibly modified by oxidation and reduction reactions with concomitant release and binding of zinc. The redox zinc switch was first described in detail in the highly conserved cytosolic heat shock protein, Hsp33 (97). The structure of the C-terminal domain of Hsp33 in its reduced form is stabilized by the high affinity coordination of zinc to two pairs of conserved cysteine residues that are far apart in primary sequence (95). Oxidation of the coordinating ligands results in the formation of an intramolecular disulfide bond between

each pair of cysteines, accompanied by zinc release and a major conformational change in the protein that activates its chaperone function (97).

Although targets for redox regulation are not limited to proteins with zinc centers, redox zinc switches are emerging, nonetheless, as a global regulation process (84) whereby oxidation/reduction signals mobilize zinc and induce diverse actions. Reactive species (RS) that have been shown *in vitro* to oxidize the sulfur donor in zinc-thiolate sites and mobilize zinc include hypoclorous acid (62) superoxide ($O_2\cdot^-$), hydrogen peroxide (H_2O_2) (63), and nitric oxide (NO) (115). Zinc may be released for the primary purpose of inducing a conformational change in the protein to which it was originally bound to alter the function of that protein, as in the activation of Hsp33, or the release of zinc itself may be the motivation for triggering the zinc switch, effectively making zinc available to interact with other proteins (133). Mammalian MT with its ability to bind up to seven zinc ions in multiple zinc containing clusters is the prototypical example of a protein that utilizes the zinc switch mechanism to release zinc to proteins that require it. This feature underscores the proposed centrality of MT to the maintenance of cellular zinc homeostasis. The regulation of MT genes involves transcription factors that themselves utilize redox zinc switches, including Sp1 and MTF-1, the latter of which also regulates gene expression of ZnT1.

Sp1 and MTF-1 are examples of proteins that contain classic Cys_2His_2 zinc finger motifs. Zinc fingers are abundant in biology and were believed to perform exclusively structural functions (219). The identification of zinc finger proteins with redox switch capabilities suggests that, in theory, all zinc fingers are susceptible to reversible oxidation. Demonstration that sulfur ligands are oxidized and released *in vivo* is technically challenging, however, and consequently little is known about the number of zinc finger proteins that are affected by redox signals under physiological conditions (133). The probability that a zinc-coordinated cysteine will be oxidized *in vivo* will depend on the physical location of the protein, the accessibility of the cysteine to the oxidant, the redox potential of the thiol group, and the antioxidant capacity of the local environment (217). Under conditions of oxidative stress, the functions of a much greater number of proteins will be compromised and it has been speculated that in such circumstances the mobilization of zinc from cysteine-coordinated binding sites by an oxidative reaction may be necessary to ensure that zinc is available for processes of antioxidant defense (28).

Antioxidant Effects of Zinc

RS, including reactive oxygen and reactive nitrogen oxide species, have important physiological roles in a wide range of signaling pathways, but their accumulation can place cells in a state of oxidative stress. Concentrations of cellular RS are therefore carefully controlled *via* a complex and coordinated antioxidant defense system. Zinc, despite being redox-inert and therefore not itself an antioxidant, exhibits a variety of indirect antioxidant effects. It has been known for some time that zinc interaction with cell membranes stabilizes them against damage (39) and that zinc enhances the antioxidant capacity of the cell through direct competition with metals that are known to catalyze the Fenton reaction, such as copper and iron. In addition, zinc is integral to the activity of superoxide dismutase (SOD) and is able to induce the synthesis of MT and glutathione, all of which protect against an accrual of RS in cellular systems.

Superoxide dismutases

SOD plays a critical role in redox metabolism by catalyzing the dismutation of the superoxide anion radical $O_2\cdot^-$ to H_2O_2 (11). In eukaryotes, three forms of SOD exist: copper, zinc SOD (CuZnSOD), which is the major intracellular SOD, present in the cytoplasm and nucleus; manganese SOD, which is located primarily in the mitochondrial matrix; and extracellular SOD (EC-SOD), the predominant SOD in extracellular fluids. Both CuZnSOD and EC-SOD require zinc for their enzymatic activity. CuZnSOD comprises two 16-kDa protein subunits, each of which incorporates one zinc ion at its cocatalytic binding site. The several isoforms of EC-SOD are tetrameric CuZnSODs, containing one zinc ion at each of the four 30-kDa subunits.

Numerous studies have demonstrated an influence of zinc status on SOD activity. In an *in vitro* model of zinc deficiency, human retinal pigment epithelium cells cultured in 0.55 μM zinc exhibited a higher increase in total SOD (but no change in manganese SOD) than cells in 14 μM zinc medium (198). It is not clear why a decrease in zinc would lead to increases in enzymes that are dependent on zinc for their activity, but it may reflect a prioritization of zinc redistribution when the zinc status of the cell is compromised that favors particular cellular processes, in this case antioxidant defense mechanisms. In support of this idea, mouse fibroblast 3T3 cells incubated in dialyzed media containing 0.5 μM or 5 μM zinc demonstrated higher levels of oxidative stress and an upregulation of oxidant defense mechanisms, including CuZnSOD activity, compared to control cells and cells cultured in 50 μM zinc (148). The results of this study should be interpreted with circumspection, however, as the authors considered the cells grown in 5 μM zinc to be zinc deficient, despite the control media having a lower zinc concentration (3.6 μM). They hypothesized that the observation of increased oxidative stress in cells grown in 5 μM zinc but not in control cells is a reflection of a zinc deficient state caused by dialysis and chelate treatment-induced changes in zinc-binding ligands. This highlights a common limitation of *in vitro* zinc studies: the amount of zinc in different media that is available to the cell is often unknown. Further, a lack of information about the total zinc content and uptake kinetics of cells under different experimental conditions makes comparisons between cell studies problematic. Even when the *in vitro* effects of zinc on particular cellular functions appear well established, it is moreover not always possible to extrapolate these effects to whole systems (28).

In animal experiments, CuZnSOD activity has been shown to be decreased by both low and high zinc intakes. In rats, a zinc-deficient diet reduced erythrocyte CuZnSOD activity and serum zinc concentrations (20, 199), whereas high dietary zinc resulted in a significant decrease in CuZnSOD activity in heart and liver tissue (118). The activity of EC-SOD, on the other hand, shows a positive association with zinc intake in animal models. Rats fed a diet supplemented with 60 mg Zn/kg demonstrated increased plasma EC-SOD activity (152), whereas dietary zinc deprivation was characterized by reduced plasma EC-SOD levels in rodent and rhesus macaque models (146).

In humans, the relationship of CuZnSOD and EC-SOD activities to zinc intake is inconsistent (Table 1). In randomized

Table 1. Effect of Zinc Supplementation, Alone or in Combination with Other Nutrients, on Copper, Zinc Superoxide Dismutase, Extracellular Superoxide Dismutase, or Total Superoxide Dismutase Activity in Healthy Humans

Reference	n *(treatment, control)*	*Participants (health description, gender, age range, or mean)*	*Trial design and duration*	*Zn dose (mg/day, anion)*	*Other nutrients*	*Effect of Zn treatment on SOD activity and plasma Zn*
Mariani *et al.* (134)	108, 0	Healthy, 53(M), 55(F), 60–92	Case series (pre- and post-test), 7 weeks	10, aspartate	—	↑ erythrocyte CuZnSOD ↑ plasma EC-SOD activity ↑ plasma [Zn]
Andriollo-Sanchez *et al.* (6), Hininger-Favier *et al.* (89)	126, 130	Healthy, 64 (M), 62 (F), 55–85	RCT, parallel, double blind, 6 months	15, gluconate	—	No overall, gender-, or age-related changes in erythrocyte CuZnSOD ↑ serum [Zn]
Girodon *et al.* (74)	20, 20	Healthy institutionalized, 29(F), 11(M), 84.2 ± 8.1	RCT, parallel, double blind, 2 years	20, sulfate	—	No change in erythrocyte CuZnSOD ↑ serum [Zn] at 6 months and 12 months (returned to baseline at 2 years)
	20, 21	Healthy institutionalized, 29(F), 12(M), 84.2 ± 8.1	RCT, parallel, double blind, 2 years	20, sulfate	Se (100 μg) + vitamins	No change in erythrocyte CuZnSOD ↑ serum [Zn] at 6 months and 12 months (returned to baseline at 2 years)
Galan *et al.* (70)	378, 378	Healthy institutionalized, 193(M), 563(F), 83.5 ± 7.6	RCT, parallel, double blind, 12 months	20, sulfate	Se (100 μg) + vitamins	No change in erythrocyte SOD ↑ serum [Zn]
Preziosi *et al.* (168)	201, 200	Healthy, 166(M), 235(F), 35–60	RCT, parallel, double blind, 6 months	20, gluconate	Se (100 μg) + vitamins	No change in erythrocyte SOD ↑ serum [Zn]
Tamura *et al.* (197)	31, 32	Healthy, pregnant, F, age not described	RCT, parallel, from 19 weeks of gestation until delivery	25, sulfate	—	↑ plasma EC-SOD in both placebo and treatment groups but no significant differences between groups No change in plasma [Zn]
Yadrick *et al.* (228)	9, 0	Healthy, F, 25–40	Case series (pre- and post-test), 10 weeks	25, gluconate	Fe (25 mg)	↓ erythrocyte CuZnSOD ↑ serum [Zn]
Andriollo-Sanchez *et al.* (6), Hininger-Favier *et al.* (89)	131, 130	Healthy, 65 (M), 66 (F), 55–85	RCT, parallel, double blind, 6 months	30, gluconate	—	No change in erythrocyte CuZnSOD ↑ serum [Zn]

Bonham *et al.* (25)	19, 19	Healthy, M, 36	RCT, parallel, double blind, 14 weeks	30, glycine chelate	—	No change in whole blood SOD No change in plasma [Zn]
Abdallah and Samman (1)	6, 0	Healthy, F, 18–36	Case series (pre- and post-test), 12 days	50, sulfate	—	↓ erythrocyte SOD activity No change in plasma [Zn]
Yadrick *et al.* (228)	9, 0	Healthy, F, 25–40	Case series (pre- and post-test), 10 weeks	50, gluconate	—	↓ erythrocyte CuZnSOD ↑ serum [Zn]
Fischer *et al.* (61)	13, 13	Healthy, M, age not specified	RCT, parallel, double blind, 6 weeks	50, gluconate	—	↓ erythrocyte CuZnSOD Inverse correlation between plasma [Zn] and erythrocyte CuZnSOD
Davis *et al.* (48)	23, 0	Healthy postmenopausal, F, 50–76	Metabolic ward study (pre- and post-test), 13 + 13 weeks	53, gluconate	Cu (1 or 3 mg)	No change in erythrocyte CuZnSOD ↑ serum EC-SOD, plasma [Zn]
Hunaiti and Saleh (93)	59, 60	Healthy pregnant, F, 15–45	Parallel, controlled trial, from first 4 weeks of pregnancy until delivery	61.8, sulfate	Fe (150 mg)	↑ placental CuZnSOD Plasma [Zn] not measured
Samman and Roberts (177, 178)	26	Healthy, F, 27	RCT, double blind, crossover, 6 + 6 weeks	150, sulfate	—	↓ erythrocyte SOD activity ↑ plasma [Zn]
	21	Healthy, M, 28	RCT, double blind, crossover, 6 + 6 weeks	150, sulfate	—	No change in erythrocyte SOD ↑ plasma [Zn]

CuZnSOD, copper, zinc superoxide dismutase; EC-SOD, extracellular SOD; RCT, randomized controlled trial.

controlled supplementation trials, no effect of zinc on CuZn-SOD was observed in healthy subjects supplemented with moderate amounts of zinc (30 mg/day or less). In contrast, zinc supplementation with 50 mg/day reduced CuZnSOD activity in men (61) and women (1), possibly by limiting the bioavailability of copper ions. Supplementation with 150 mg Zn/day, however, decreased CuZnSOD activity in healthy women but not in men (177). In relation to EC-SOD activity, augmented zinc intakes increased the enzyme activities in two recent studies in healthy individuals (48, 134). Conversely, supplementation with 25 mg Zn/day did not influence the activity of plasma EC-SOD in healthy pregnant African American women compared to controls (197) and a cross-sectional study found no correlation between plasma EC-SOD activity and dietary zinc intake (150), again indicating that the relationship between zinc supplementation and SOD activity in humans is far from obvious. Diet-induced marginal zinc deficiency has been associated with a significant lowering of erythrocyte CuZnSOD activity in healthy males, whereas plasma EC-SOD activity decreased during the first 6 weeks of zinc depletion but then increased in the final week of depletion and in the first week of repletion before finally decreasing again (175). The significance of these changes is unclear. A possible explanation is that the varying responses reflect priorities of tissue zinc redistribution in response to dietary zinc fluctuations.

Metallothionein

MTs comprise a class of ubiquitous, low-molecular-weight proteins that participate in cellular zinc distribution and homeostasis, contribute to the antioxidant defense system by scavenging RS, and are critically involved in redox and zinc signaling. Although primarily a cytoplasmic protein, MT can translocate to the intermembrane space of mitochondria (230) or to the nucleus (36, 187) and has been reported to occur in low levels in extracellular fluids (179).

Human MTs have 60–68 amino acids, 20 of which are conserved cysteine residues that have the capacity to bind a maximum of four zinc ions in the C-terminal α domain (utilizing the complementarity of zinc and the sulfur donors of 11 cysteines) and three ions in the N-terminal β domain (nine cysteines) in zinc-thiolate clusters. There are at least three classes of cysteine-coordinated zinc binding sites, each of which binds zinc with a different affinity (130). Although the zinc ions are embedded within the conformational structure of the MT protein, as discussed previously the zinc-bound thiolate ligands are available to participate in redox reactions with concomitant release and binding of zinc, linking the control of the metal content of MT to the redox status of the cellular environment (131). MT gene expression is usually inducible. Stimuli that have been shown to induce MT expression include zinc and other heavy metals, hormones, cytokines, shifts in glutathione redox status, and a variety of physical, chemical, and oxidative stresses. MT was induced in rat liver and kidney after exposure to cold, heat, burn, strenuous exercise, and carbon tetrachloride administration (145). In mice, oxidative stress has been shown to activate the DNA-binding activity of MTF-1 and increase MT expression (47). In the majority of cell culture and *in vivo* experiments, MT induction is associated with protection against subsequent metal, chemical, and other stresses (201, 203).

Zinc supplementation increases and zinc depletion decreases MT mRNA and protein levels in cell cultures (31) and in the vast majority of animal and human zinc intake studies. In rats, dietary zinc has been shown to increase and zinc deficiency to decrease MT levels in a variety of tissues, including the pancreas, liver, intestine, kidney, and blood (29, 147, 196). A similar response pattern of MT to zinc has been observed in healthy men (3, 200). The effect of zinc supplementation on MT expression in women, however, has received little attention. More studies are also needed in disease states; in men and women with inflammatory bowel disease, supplementation with 60 mg Zn/day did not result in any significant changes in the concentration of plasma or erythrocyte MT, despite an increase in plasma zinc.

Glutathione

The most abundant antioxidant in eukaryotic cells is glutathione (γ-glutamyl-cysteinyl-glycine; GSH), a thiol-containing tripeptide that is central to the maintenance of the cellular redox state and the essential thiol status of proteins (123). The antioxidant functions of GSH include the direct scavenging of RS, as well as its participation as an essential cofactor in reactions catalyzed by antioxidant enzymes, such as glutathione peroxidase (41). Roles for GSH have been identified also in the regulation of such critical cellular processes as signal transduction, DNA synthesis, cell proliferation, and immune function.

The total cellular GSH concentration is determined by the rate of GSH *de novo* synthesis, the cellular capacity to recycle GSH, and the efflux of glutathione disulfide, the oxidized form of GSH, from the cell (41). One of the major determinants of the level of GSH *de novo* synthesis is the activity of the rate-limiting enzyme glutamate cysteine ligase (GCL), a heterodimer composed of a catalytic (heavy) subunit (GCLC) and a modifier (light) subunit (GCLM). Zinc has been shown to influence the expression of GCL and thereby modulate the total cellular GSH concentration. In cultured human retinal pigment epithelial cell line ARPE-19 cells, supplementation with zinc concentrations of 100–150 μM was reported to upregulate the mRNA levels of both GCLC and GCLM *via* an ARE-nuclear redox factor 2 (Nrf2)-dependent pathway (79). Similarly, supplementation of primary rat endothelial cells with 100–200 μM zinc, being concentrations determined to be nontoxic by cell viability experiments, generated significant Nrf2-dependent increases in GCLC mRNA, GCLC protein, and total cellular GSH levels that were associated with protection against H_2O_2-induced toxicity (40). Conversely, cells depleted of intracellular zinc by means of a noncytotoxic concentration of *N*,*N*,*N*′,*N*′-tetrakis-(2-pyridylmethyl)-Ethylenediamine (TPEN) (3.3 μM) demonstrated decreased GCLC mRNA and protein concentrations, with a concomitant reduction in GSH levels, compared to untreated cells. The addition of equimolar amounts of zinc completely abrogated the TPEN effects, confirming that they resulted primarily from the chelation of zinc (40).

Zinc and Redox Signaling

In addition to its antioxidant-related roles in controlling the concentrations of cellular RS, zinc modulates the functions and protein–protein interactions of numerous redox-sensitive proteins at several levels of signaling cascades. Moreover, it has been suggested that zinc itself acts as a signaling ion and

may function to extend the signaling capacity of calcium and magnesium, the other redox-inert metal ions involved in redox metabolism, to cover not only millimolar and micromolar but also nanomolar concentrations (129). A number of select examples of the effects of zinc on redox-sensitive signaling processes serve to highlight the importance of zinc in cellular function and communication.

Transcription factors—the example of nuclear factor-kappa B

Zinc is amphoteric and has the ability to form flexible coordination geometry, both characteristics that contribute to its biological versatility (210). One of the ways in which zinc is involved in cell signal transduction is *via* its influence on the activity of transcription factors, such as the oxidant-responsive nuclear factor-kappa B (NF-κB). Unlike examples of zinc-regulated transcription factors that have zinc–sulfur coordination sites, such as MTF-1 and p53, but similar to the zinc-responsive AP-1 leucine zipper, NF-κB is not itself a zinc protein. In its classic form, NF-κB is a heterodimer of the p65/Rel A and p50 subunits. In unstimulated cells, it resides in the cytoplasm complexed to the inhibitor of NF-κB (IκB). The canonical pathway of NF-κB activation involves phosphorylation of IκB by IκB kinase with the concomitant release of NF-κB, which is then able to translocate to the nucleus and bind to target gene promoters. NF-κB is ubiquitously expressed and impacts an extensive assortment of cellular processes, including proliferation, immunity, inflammation, and apoptosis.

NF-κB binding has been shown *in vitro* to be inhibited by oxidation of critical cysteine residues (204). In contrast, DNA binding and transactivation of NF-κB were strongly activated by H_2O_2 in a subsequent study (140). Zinc has been depicted as both a negative and positive regulator of NF-κB. *In vivo*, the effects of zinc on NF-κB activity appear to depend on the health and/or zinc status of the host. Diet-induced zinc deficiency in a murine model of polymicrobial sepsis enhanced NF-κB p65 DNA binding activity in vital organs and expression of a range of NF-κB-targeted genes known to increase systemic inflammation. Short-term zinc repletion before the onset of sepsis significantly reduced these effects (14). In humans, NF-κB activation and the mRNA levels of the NF-κB-regulated interleukin (IL)-2 cytokine and IL-2Rα receptor were found to be decreased in the peripheral blood mononuclear cells of elderly subjects with plasma zinc levels below the reference range. These effects were corrected with zinc supplementation of 45 mg/day Zn gluconate (166). The modulatory effects of zinc on NF-κB activity *in vivo* may also vary according to the study model. In mice with chemically induced diabetes, zinc supplementation inhibited the activation of pancreatic NF-κB and thereby prevented inflammatory immune reactions, whereas in immune cell-mediated diabetic mice the transactivation of NF-κB in response to zinc protected against apoptosis (181).

In vitro, incubation of human HUT-78 (Th_0) cells in media containing either low (1 μM) or high (50 μM or 100 μM) zinc levels decreased the activation of NF-κB and the expression levels of IL-2, IL-2R, and tumor necrosis factor (TNF)-α compared to cells grown in 15 μM zinc medium. Cell growth (but not cell viability) was observed to be lower in the 1, 50, and 100 μM zinc media, suggesting an altered cellular metabolism in cells exposed to non-physiological concentrations of extracellular zinc (13). In human monocytes, 10, 20, and 45 μM zinc/pyrithione (50 μM) decreased the lipopolysaccaride-induced activation of NF-κB in a concentration-dependent manner; it was found that zinc-mediated cyclic guanosine monophosphate (cGMP) elevation cross-activated protein kinase A, resulting in inhibitory phosphorylation of Raf-1, whereupon protein kinase A intervened in the Raf-1 pathway and inhibited NF-κB translocation to the nucleus (213). In contrast, exposure of cultured human airway epithelial cells to 50 μM of zinc increased NF-κB-dependent transcriptional activity, seemingly *via* the zinc-induced phosphorylation of multiple p65 serine residues with phosphorylation of Ser 563 by IκB kinase being pivotal (104). The incubation of human neuroblastoma IMR-32 cells in media containing 1.5 and 5 μM zinc concentrations has been associated with low intracellular zinc levels (compared to cells in 15 and 50 μM zinc media) and an increased activation of NF-κB; however, the active dimer demonstrated an altered nuclear translocation, appearing to accumulate in the cytosol, and the zinc-deficient cells exhibited decreased cell viability (124). Differences in cell model, the zinc concentrations used, and the impact of various agents (such as chelating agents and zinc ionophores) on intracellular free zinc fluctuations make apparently contradictory *in vitro* observations of the effect of zinc on NF-κB activation difficult to reconcile.

Protein phosphorylation

As illustrated above by the proposed mechanisms of NF-κB regulation by zinc ions, zinc appears able to modulate phosphorylation signaling. In principle, increased protein phosphorylation is caused by activation of kinases or inhibition of phosphatases. The serine/threonine kinases protein kinase C (PKC) and Raf, mitogen activated protein kinases (MAPKs), protein tyrosine phosphatase (PTP), and the phosphatase and tensin homolog (PTEN) lipid phosphatase are all examples of enzymes that have been shown to be zinc responsive.

Intracellular protein phosphorylation by PKC plays a major role in the translation of extracellular signals into cellular events. PKC is a redox-sensitive zinc-protein; $O_2\cdot^-$ has been shown to oxidize the thiol groups of cysteine-coordinated zinc sites in the regulatory domain of the enzyme, causing zinc to be released and PKC activity to be stimulated (112). A regulatory function of zinc for PKC was inferred initially by the observation *in vitro* that zinc can activate PKC and induce its translocation to the plasma membrane (45). An earlier study that found no effect of zinc on protein kinase M (96), a proteolytic product of PKC that lacks its regulatory domain and hence the zinc–sulfur binding sites, suggests that the zinc binding sites may mediate the putative regulatory effects of zinc on PKC. This idea has been disputed (21); PKC-bound zinc was unable to be removed even by high affinity heavy metal ion chelators (91), whereas chelation by TPEN (45) and 1,10-phenanatroline (64) resulted in the inhibition of PKC activation, suggesting that PKC activation is regulated by a chelatable pool of zinc that is not identical to the zinc bound within the zinc–sulfur coordination sites of the enzyme.

In a study of the effects of zinc deficiency on PKC processing and activity, 3T3 cells cultured in a zinc-deficient (0.5 μM Zn) medium exhibited decreased zinc content, lowered cytosolic classical PKC-α activity, and reduced cell number. Zinc

depletion additionally resulted in the appearance in mitochondria of the 40-kDa catalytically active fragment of PKC-δ, which, in contrast to the functions of classical PKC isoforms in cell survival, is involved in pro-apoptotic signaling pathways. Addition of zinc or the PKC-δ inhibitor, rottlerin, to the zinc-deficient medium reduced or eliminated proteolysis of PKC-δ and restored cell number (38). While these results appear to support the concept that intracellular zinc concentrations influence PKC activity, it remains unclear whether the effects of zinc depletion on PKC are directly related to zinc or occur as a result of other factors affecting cell viability, such as an underlying increase in oxidative stress.

Members of the Raf family of serine/threonine kinases, which form part of the Ras/Raf/mitogen-activated kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway, show structural similarity to PKC and also contain zinc–sulfur sites in their regulatory domains. Data derived from *Xenopus* oocytes and cultured mammalian cells suggest that Raf-1 activity is promoted by the binding of cation diffusion facilitator (CDF)/ZnT-1 *via* its carboxyl-terminal segment to the Raf-1 regulatory domain, an interaction that was inhibited by zinc (99). The same group had previously shown that overexpression of CDF-1 or ZnT-1 in *Xenopus* oocytes promoted ERK MAPK activation, while an elevated zinc concentration suppressed ERK activation (30). In other words, this model suggests that CDF proteins, which promote zinc export from mammalian cells, enhance signaling by lowering the concentration of intracellular zinc and that signaling is diminished when the concentration of zinc is raised. The authors hypothesize that, upon binding to Raf-1, CDF proteins may remove zinc ions from the Raf-1 regulatory domain, thereby facilitating the dissociation of the amino-terminal segment of Raf-1 from the carboxyl-terminal domain of CDF and stimulating Raf-1 kinase activity (99).

ERK, which occurs downstream from Raf, and its MAPK family members c-Jun N-terminal kinase (JNK) and p38, are further examples of enzymes that can be modulated both by oxidants and zinc. The treatment of vascular smooth muscle cells with H_2O_2 has been shown to stimulate ERK and p38 phosphorylation in a dose- and time-dependent fashion (23). In zinc-deficient human IMR-32 cells, which exhibit increased cell oxidants and H_2O_2 release, an H_2O_2-dependent increase in JNK and p38 activation was observed, along with an H_2O_2-independent reduction in the levels of activating ERK phosphorylation (231). The decrease in ERK phosphorylation in zinc-deficient cells was accompanied by a reduction in cell proliferation (231), which accords with a role for ERK in cell proliferation-related signaling and may be related to the requirement for zinc in the proliferation of mammalian cells (21).

In zinc supplementation studies, when increasing concentrations of zinc (1–100 μmol/L) were administered to rat glioma cells in combination with the ionophore pyrithione (50 μmol/L), only lower concentrations of supplemented zinc (2.5 and 10 μmol/L) were shown to activate ERK, whereas higher concentrations (50 and 100 μmol/L) reduced the activating phosphorylation (80). In contrast, exposure of murine cortical cultures for 30 min to 300 μmol/L zinc, which induced gradually developing neuronal death, was found to stimulate sustained ERK activation (154). In human airway epithelial cells, zinc exposure induced the phosphorylation of ERK as well as JNK and p38 MAPKs and significantly retarded the dephosphorylation rate of recombinant phospho-ERK and phospho-JNK (105). Similarly, inhibition of ERK and p38 dephosphorylation was demonstrated in zinc-treated human monocytes (82). These latter two studies suggest an inhibitory effect of zinc on the dual-specificity MAP-kinase phosphatases, which negatively regulate MAPK activity *via* dephosphorylation of tyrosine, serine/threonine, or both tyrosine and threonine MAPK residues (105).

The inhibition of phosphatases by zinc is further illustrated by the ability of zinc to activate the phosphoinositide 3′-kinase (PI3K)/Akt signaling pathway (15), which, among its many roles, constitutes an archetypal stress-response cascade and is involved in insulin and leptin signaling. The classic type Ia PI3Ks catalyze the conversion of phosphatidylinositol-3,4-bisphosphate to phosphatidylinositol-3,4,5-trisphosphate, which in turn recruits phosphoinositide-dependent kinases and Akt (also known as protein kinase B) to the cell membrane, thus facilitating the activation of Akt by phosphoinositide-dependent kinase-mediated phosphorylation. Zinc has been shown to attenuate the activity of redox-sensitive protein tyrosine phosphatases, such as PTP1B, which otherwise act to inhibit the PI3K-dependent activation of Akt, and lipid phosphatases, such as PTEN, which negatively regulate the PI3K/Akt pathway by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (15, 116) (Fig. 1). In its active form, Akt facilitates important cellular responses to growth factors and oxidative stress and promotes cell survival by phosphorylating and reducing the activity of proapoptotic factors such as caspase-9. Downstream, a number of the forkhead box (FoxO) transcription factors are directly targeted by Akt, which inactivates them *via* phosphorylation. FoxO proteins are potent transactivators of genes involved in glucose metabolism and their inactivation has been shown to mediate the inhibition of hepatic glucose production by insulin (116). They have also been ascribed a number of roles in lipid metabolism (233).

NO signaling

NO is an endogenous signaling molecule that is synthesized from L-arginine and O_2 by members of the NO synthase (NOS) family of dimeric enzymes. The redox-signaling roles of NO are related to zinc in at least two ways. First, the zinc-dependent enzymes CuZnSOD and EC-SOD function to protect the cellular availability of NO by controlling $O_2^{\bullet -}$ levels, which if left unchecked react with NO to form $ONOO^-$. The $ONOO^-$ anion in turn has been shown to oxidize the zinc–thiolate cluster at the dimer interface of endothelial NOS (eNOS), leading to the release of zinc and consequent dimer disruption and uncoupling of the enzyme; uncoupled eNOS demonstrates increased $O_2^{\bullet -}$ production and decreased NO synthesis (235) (Fig. 2). Second, NO is known to interact with the cysteine residues of MT, inducing a conformational change in the protein and a concomitant release of zinc (115, 157). MT thionitrosation may be reversed by additional zinc ions (42). It has been suggested that the NO-mediated zinc release from MT provides a novel mechanism for NO-based signaling through the regulation of zinc homeostasis (190). In addition, NO derived from inducible NOS (iNOS) was found in one study to induce the transient increase of zinc ions almost exclusively within cell nuclei, an event that positively correlated with the NO-mediated nuclear translocation of MT and implicates MT as the essential carrier molecule in this pathway (187). The functional relevance of the nuclear appearance of labile zinc

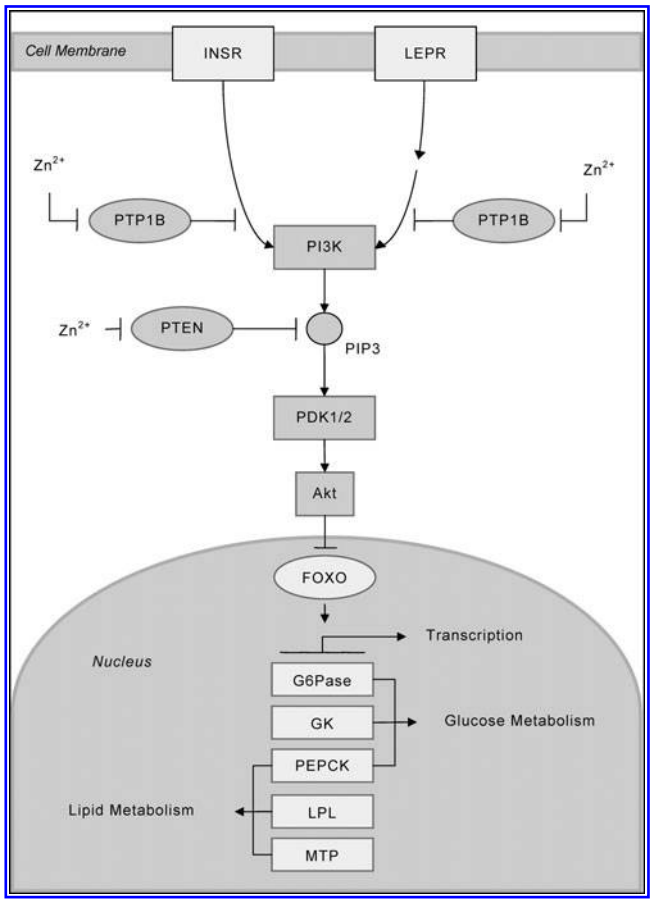

FIG. 1. The ability of Zn^{2+} to induce the classical PI3K/Akt pathway influences insulin and leptin signaling. The PI3K/Akt pathway is a major mediator of both insulin and leptin signaling. Ligand binding to the INSR or LEPR stimulates PI3K, which leads to the production of PIP3. PIP3 activates 3-PDK 1/2, which in turn phosphorylates and thereby activates the serine/threonine kinase Akt, enabling it to translocate to the nucleus and mediate the inhibition of the FoxO transcription factors. The Akt-mediated suppression of FoxO activity prevents FoxO from transactivating various target genes involved in glucose and lipid metabolism, including G6Pase, GK, PEPCK, LPL, and MTP. Zinc is able to activate the insulin and leptin-sensitive PI3K/Akt signaling pathway *via* inhibition of either protein tyrosine phosphatases (*e.g.*, PTP1B), which otherwise act to inhibit the PI3K-dependent activation of Akt, or lipid phosphatases (*e.g.*, PTEN), which negatively regulate the PI3K/Akt pathway by dephosphorylating and inactivating PIP3. In glucose-related signaling, PTPase is the key inhibitor targeted by zinc, while the inactivation of FoxO in lipid metabolism appears instead to be modulated by PTEN. FoxO, forkhead box; G6Pase, glucose-6-phosphatase; GK, glucokinase; INSR, insulin receptor; LEPR, leptin receptor; LPL, lipoprotein lipase; MTP, microsomal triglyceride transfer protein; PDK, phosphoinositide-dependent protein kinase; PEPCK, phosphoenolpyruvate carboxykinase; PI3K, phosphoinositide 3′-kinase; PIP3, phosphatidylinositol-3,4,5-triphosphate; PTEN, phosphatase and tensin homolog; PTP, protein tyrosine phosphatase.

has yet to be determined, although it is conceivable that the released zinc could interact with a number of zinc-dependent transcriptional and signaling pathways, including NF-κB-mediated expression of iNOS itself.

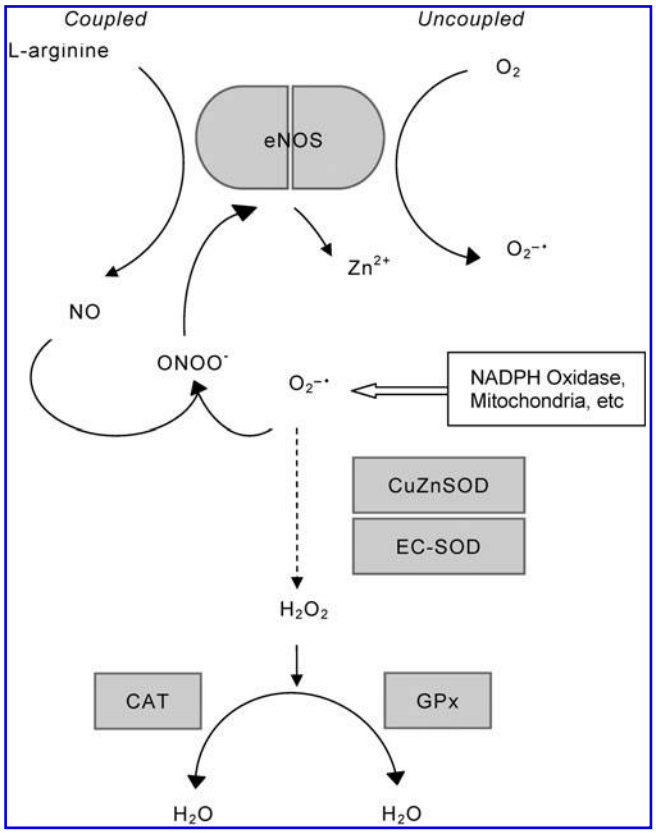

FIG. 2. Reductions in CuZnSOD and EC-SOD may contribute to eNOS uncoupling. The superoxide radical $O_2^{\cdot -}$ is generated in a number of ways *in vivo*, including *via* the mitochondrial electron transport chain and during the normal catalytic function of NADPH oxidase and other enzymes. The zinc-dependent antioxidant enzymes CuZnSOD and EC-SOD protect the cell against an accumulation of $O_2^{\cdot -}$ by catalyzing the dismutation of the superoxide radical $O_2^{\cdot -}$ to H_2O_2, which is then converted to H_2O by CAT or GPx. If the activities of CuZnSOD and EC-SOD are reduced (indicated by the dashed arrow), increasing amounts of $O_2^{\cdot -}$ are at liberty to react with NO to form $ONOO^-$. The $ONOO^-$ radical has been shown to oxidize the zinc-thiolate cluster at the dimer interface of eNOS, affecting the stability of the eNOS dimer and resulting in zinc release and uncoupling of the enzyme. Uncoupled eNOS generates further increases in $O_2^{\cdot -}$ (rather than NO) production. CAT, catalase; CuZnSOD, copper, zinc SOD; EC-SOD, extracellular superoxide dismutase; eNOS, endothelial nitric oxide synthase; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; NADPH, nicotinamide adenine dinucleotide phosphate; $O_2^{\cdot -}$, superoxide.

Zinc and calcium interactions

While it is clear that zinc influences numerous signaling molecules involved in redox metabolism and cellular stress responses, there are some recent indications that zinc itself acts as a potent biological signal. Zinc ions show a number of similarities in behavior to calcium, a redox-inert metal ion known to be directly involved in redox metabolism, and crosstalk has been observed between zinc and calcium signaling. Differing concentrations of zinc ions have been demonstrated to interfere with calcium/calmodulin signaling (121) and the elevation of extracellular zinc has been found to evoke intracellular calcium mobilization by stimulation of

hormone-sensitive intracellular calcium stores in hepatocytes (138). In the colonocytic cell line HT29, a micromolar concentration of extracellular zinc was shown to trigger the release of intracellular calcium ions from thapsigargin-sensitive stores in a manner that was dependent on the binding of inositol 1,4,5-triphosphate to its receptor (87). This lead to the suggestion that a Gq-coupled, zinc-sensing receptor (the putative ZnR receptor) exists that is capable of linking changes in extracellular zinc with downstream signal transduction pathways, including those of MAPK and PI3K, and thereby of mediating key cellular functions (88). In addition to the effect of extracellular zinc on calcium concentrations, intracellular calcium may affect zinc distribution *via* its ability to induce eNOS-generated NO, which in turn can react with MT and result in the release of bound zinc (157). Moreover, in immunoglobulin E-sensitized murine mast cells a calcium-dependent release of intracellular zinc, coined the zinc wave, was observed in the area of the endoplasmic reticulum (191), again supporting the proposition that zinc and calcium signaling overlap. Although it is not yet apparent whether zinc can strictly be defined as a second messenger in the manner of calcium, interactions between zinc and calcium signaling and their impact on redox metabolism pose interesting future avenues of exploration in zinc biology.

Zinc Deficiency, Oxidative Stress, and Chronic Disease

Given the interconnectedness and widespread effects of cellular zinc- and redox-dependent signal transduction pathways, it is perhaps unsurprising that perturbations in zinc homeostasis appear to exacerbate oxidative stress. Although both zinc deficiency and zinc overload can lead to a rapid increase in cellular RS, the deficiency state is more prevalent in human populations and has been linked to a number of chronic diseases, including CVD and DM. There is growing interest in the potential for nutritional zinc supplementation to ameliorate oxidative stress in these disorders.

Zinc deficiency

Globally, zinc deficiency is a significant contributor to the burden of disease (226). Severe zinc deficiency is characterized by impaired growth, delayed sexual and bone maturation, hypogonadism, impaired immunity including infections of the epithelium and poor wound healing, diarrhea, and decreased appetite. This pathophysiology is evident in acrodermatitis enteropathica, an autosomal recessive disorder of intestinal zinc malabsorption. The etiology of acrodermatitis enteropathica has been linked to mutations in the *ZIP4* gene (216), a key transporter involved in zinc absorption/reabsorption from the gastrointestinal tract, highlighting the importance of zinc transporters in zinc homeostasis.

Although severe zinc deficiency is relatively rare in developed countries, less acute deficiency states are believed to be highly prevalent. The most obvious factor attributed to the development of zinc deficiency is an inadequate intake of dietary zinc, often in conjunction with high intakes of phytic acid, a potent inhibitor of zinc absorption. Using national food balance data obtained from the Food and Agriculture Organization of the United Nations for 176 countries, it has been estimated that approximately 20% of the world's population is at risk of inadequate zinc intake (226). Deficiencies associated with low intakes of absorbable zinc are exacerbated during times of increased requirement, including growth, pregnancy, and lactation.

Early manifestations of zinc deficiency are nonspecific and the determination of zinc deficiency is made difficult by the lack of a sensitive and reliable biochemical marker of zinc status. Currently, the most commonly used biomarker is plasma zinc; however, the effectiveness of homeostatic mechanisms in maintaining plasma zinc concentrations within defined limits (10–18 μmol/L), even in the presence of dietary zinc restriction (141), renders it an insensitive marker of the zinc status of an individual. While clinical symptoms of zinc deficiency do not become evident until after the plasma zinc concentration has fallen to 4 μmol/L (108), the effects of zinc deficiency on specific cellular functions appear to occur before plasma zinc falls below the normal range. In particular, aspects of cell-mediated immunity are suppressed, including serum thymulin activity, recruitment of autoreactive T-cells, and NK cell lytic activity (164).

In addition to diet-induced zinc deficiency, pathologic conditions may cause or contribute to a zinc deficient state. Gastrointestinal disorders can result in reduced intestinal absorption and/or reabsorption of zinc. Conditions such as chronic diarrhea, excessive burns, or traumatic and surgical wounds increase endogenous zinc losses, while numerous chronic diseases, including CVD and DM, have been associated with impaired zinc utilization resulting in a deficiency state.

Cardiovascular disease

CVD encompasses a diverse range of disorders of the heart and blood vessels. Examples from atherosclerosis, one of the primary underlying causes of CVD, and hypertension, one of its major preventable risk factors, illustrate the potential interplay between zinc deficiency and oxidative stress in cardiovascular disorders.

Atherosclerosis. An altered distribution of zinc among its principal plasma proteins has been observed in atherosclerosis (126) and the ease with which labile zinc is transported into endothelial cells (174) suggests that the vascular endothelium may be particularly affected by perturbations in zinc homeostasis and metabolism (17). Indeed, a number of the characteristic features of atherosclerosis are influenced by zinc, including enhanced apoptosis, disturbed NO and NF-κB-related signaling mechanisms, and the oxidative modification of low-density lipoprotein (LDL).

The induction of endothelial cell apoptosis in response to conditions of oxidative stress is a typical atherogenic trait. Zinc deficiency has been suggested to exacerbate the detrimental effects of specific fatty acids, such as linoleic acid, and inflammatory cytokines, such as TNF-α, on vascular endothelial functions. Endothelial cells rendered zinc deficient by exposure to the membrane-permeable chelator TPEN demonstrated considerably higher levels of apoptotic cell death and caspase-3 activity than control cells when stimulated with linoleic acid and TNF-α. This effect was completely blocked by concurrent administration of physiological amounts of zinc to the culture medium (139). Conversely, increases in intracellular free zinc levels induced by H_2O_2-stimulation resulted in a significant rise in oxidative-stress-related apoptosis of endothelial cells (223), highlighting that a change in the cellular

zinc concentration in either direction can promote cell death in the endothelium.

Aberrant expression of NF-kB is another common feature of atherosclerosis. Cellular zinc deficiency has been shown to upregulate NF-kB activity in endothelial cells (85) and high levels of NF-kB have been found to be present in the smooth muscle cells of the atherosclerotic lesion (27). NF-kB is a key component of the adhesion molecule upregulation process, is involved in the promotion of smooth muscle cell proliferation (49), and mediates signal transduction by toll-like receptors, which play an important role in the initiation of the innate immune response and are implicated in the development and progression of atherosclerotic disease (156).

The release of NO by the endothelium plays a key role in vascular homeostasis. As mentioned previously, the presence of high levels of $O_2\bullet^-$ can suppress the concentration of NO by reacting with it to form $ONOO^-$, which in turn has been shown to oxidize the zinc–thiolate cluster of eNOS, promoting uncoupling of the enzyme and a further increase in $O_2\bullet^-$ levels (Fig. 2). Numerous studies have reported that eNOS uncoupling is an important mechanism of pathologic $O_2\bullet^-$ production in the vascular endothelium (202). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase also has been proposed to play a central role in eNOS uncoupling (227). Increased expression of the $p22^{phox}$ subunit of NADPH oxidase has been demonstrated in the walls of human coronary atherosclerotic arteries (10), and NADPH oxidase appears able to be activated by zinc (137).

Another means by which the relationship between NO and zinc may promote the progression of atherosclerosis relates to Nrf2 expression in vascular cells, which is a key factor in the cellular protection against oxidative stress and inflammation. A release of intracellular zinc from proteins containing zinc–sulfur complexes, stimulated by inducible NOS-derived NO, has been shown to be a critical component of an Nrf2-dependent signaling pathway that activates the GSH redox cycle in endothelial cells, ultimately protecting against oxidative damage (41). Localized zinc deficiency or the potential for zinc to be aberrantly redistributed among target proteins and intracellular compartments during the atherosclerotic process likely ameliorates this protective effect.

Atherosclerosis typically incorporates increased levels of oxidized lipids and lipoproteins in the vessel wall (193). Oxidized LDL (oxLDL) in particular has been demonstrated to play a critical role in abnormal endothelial vasorelaxation (191) and excess uptake of oxLDL can disrupt the endothelium, injuring endothelial cells or committing them to apoptosis. Zinc has been found to inhibit the formation of oxLDL (172, 220) and zinc deficiency to enhance LDL oxidation *in vitro* (53), again indicative of an interaction between perturbed zinc levels and oxidative processes in atherosclerosis. Few studies, however, have explored the effects of zinc on LDL oxidation in humans. In healthy men supplemented with 50 mg Zn/day, no significant changes were observed in LDL oxidation levels (71), and supplementation with 15 or 30 mg Zn/day had no effect on *in vitro* LDL oxidation parameters in healthy subjects aged 55–70 years (60). The effects in humans of varying zinc doses on oxLDL concentrations in disease states are largely unknown.

Essential Hypertension. Recent studies have confirmed a role for changes in the distribution of zinc between the extracellular and intracellular spaces in the pathogenesis of arterial hypertension (206) and various degrees of zinc deficiency have been associated with the disorder. Analysis of the National Health and Nutrition Examination Survey II data revealed significantly lower serum zinc levels in older hypertensive women and men with isolated systolic hypertension (83), which may result from lower dietary zinc intake, increased urinary zinc excretion, or redistribution of zinc into other tissue compartments, such as red blood cells (69). Serum zinc levels also have been found to be lower in younger persons with hypertension, although the decrease did not reach statistical significance (83). A number of mechanisms have been proposed to explain the effects of altered zinc levels in the disease, many of which also impact redox metabolism, such as changes in SOD activity, an impaired vascular NO system, and leptin signaling effects.

In spontaneously hypertensive rats, a reduction in CuZnSOD activity in the thoracic aorta was observed in diet-induced zinc deficiency (180) while zinc supplementation resulted in significantly increased CuZnSOD activity (51). In human hypertension, inverse correlations have been observed between diastolic blood pressure and CuZnSOD (212) and reductions in EC-SOD also have been noted (234). A compromised ability of CuZnSOD and EC-SOD to assist in controlling the cellular $O_2\bullet^-$ concentration would help to explain the increased levels of $O_2\bullet^-$ that have been associated with the pathogenesis of hypertension. High levels of $O_2\bullet^-$ react with NO to form $ONOO^-$, an oxidant that has been demonstrated to attack the zinc-thiolate cluster of eNOS and thereby cause uncoupling of the enzyme and an increase in $O_2\bullet^-$ (rather than NO) production (Fig. 2). Increased $O_2\bullet^-$ production in conjunction with diminished NO generation has been identified in phorbol myristate acetate (PMA)-stimulated mononuclear cells from hypertensive patients (65) as has evidence for eNOS uncoupling (142).

Alterations in zinc homeostasis also have been suggested to contribute to hypertension by impacting leptin-sensitive pathways, which include the ability of leptin to promote NO production. Leptin is believed to activate eNOS by a serine/threonine kinase Akt-dependent mechanism similar to that of insulin. Akt-dependent insulin signaling has been shown to be inducible by zinc (Fig. 1), suggesting that zinc may interact with the leptin signaling pathway in a related manner (211). It is possible that zinc may also impact leptin signaling *via* a direct influence on leptin concentrations. In both rats and healthy humans, leptin levels have been shown to respond to changes in cellular zinc concentrations, with zinc deficiency decreasing and zinc supplementation increasing leptin concentrations (127, 128). A positive relationship between zinc and leptin suggests, however, that a zinc-deficient state in hypertension would likely lead to a reduction in leptin concentrations, whereas elevated plasma leptin has recently been demonstrated in patients with essential hypertension (19). Nonetheless, the nature of any direct relationship between zinc and leptin in disease states is unlikely to be predictable and is thus worthy of further exploration.

A putative role for nutritional zinc supplementation in CVD

The suggestion that zinc supplementation could provide a possible adjunct therapy for a range of cardiovascular

disorders is compelling, particularly where zinc intake is deficient or zinc homeostasis is shown to be perturbed. Longitudinal studies describe the involvement of zinc deficiency in CVD mortality in the population generally (169, 184) as well as in cohorts at substantial risk of future cardiovascular events (160), including those with type 2 DM (186). These studies are supported by a large prospective cohort study showing an inverse association between dietary zinc intake and CVD mortality among postmenopausal women who consume at least 10 g alcohol per day (119). In older patients with congestive heart failure, zinc intakes appear to fall short of recommended levels (76), while reduced zinc levels were recently reported in the peripheral blood mononuclear cells of patients with atherosclerosis (73). In chronic cardiac failure, hyperzincuria accompanied by low plasma and erythrocyte zinc levels has been detected in patients with dilated cardiomyopathy, whereas plasma and erythrocyte zinc levels were depressed without increased zincuria in subjects with hypertrophic cardiomyopathy (171).

In vitro and *in vivo* animal studies support a cardioprotective effect of zinc (161). Zinc was shown, for example, to reduce catecholamine-induced cardiac oxidative injury (158, 183) and preserve postischemic function in models of cardiac ischemic injury (162, 163). Hearts from rats receiving dietary supplementation of the zinc ionophore pyrithione recovered fully from ischemia/reperfusion, *via* a mechanism that was reported to involve the zinc-mediated protection from degradation of PKC isoforms (101). Zinc supplementation of mice with chemically-induced diabetes was found to protect against diabetic cardiomyopathy, an effect that was purportedly mediated by cardiac MT induction (215). Despite indications that zinc supplementation may be beneficial in CVD, studies exploring the effects of zinc nutrition on cardiovascular outcomes and risk factors in humans are few and inconsistent. Acute zinc depletion of healthy males has been found to impair platelet aggregation and prolong bleeding times (75), supporting a role for zinc in hemostasis (92). On the other hand, supplementation with 50 mg Zn/day has been shown to increase platelet reactivity (136), and at lower doses of zinc (30 mg) fibrinolytic factors and platelet zinc concentrations are unaffected (24, 25, 175, 195). In a meta-analysis of controlled trials, a significant decrease in plasma HDL cholesterol concentrations was observed in healthy individuals (Fig. 3), equivalent to a 7% decrease from baseline (67). In contrast, an increase in plasma HDL cholesterol concentrations was observed in subjects with type 2 DM (Fig. 3), suggesting that zinc supplementation has a beneficial effect on CVD risk only in those with an underlying perturbation in zinc homeostasis. In a randomized controlled placebo trial in 40 ostensibly healthy elderly subjects, supplementation with 45 mg Zn/day for 6 months was associated with an increase in antioxidant power and a decrease in plasma concentrations of C-reactive protein, IL-6, macrophage chemo-attractant protein 1, vascular endothelial cell adhesion molecule 1, and oxidative stress markers, indicating that zinc has an atheroprotective effect in this population (12). It is conceivable that zinc supplementation addressed an underlying perturbation in zinc homeostasis, given that elderly populations are believed to be susceptible to mild to moderate zinc deficiency (167).

The supposition that the effect of zinc supplementation on CVD risk is dependent on zinc status is supported by studies

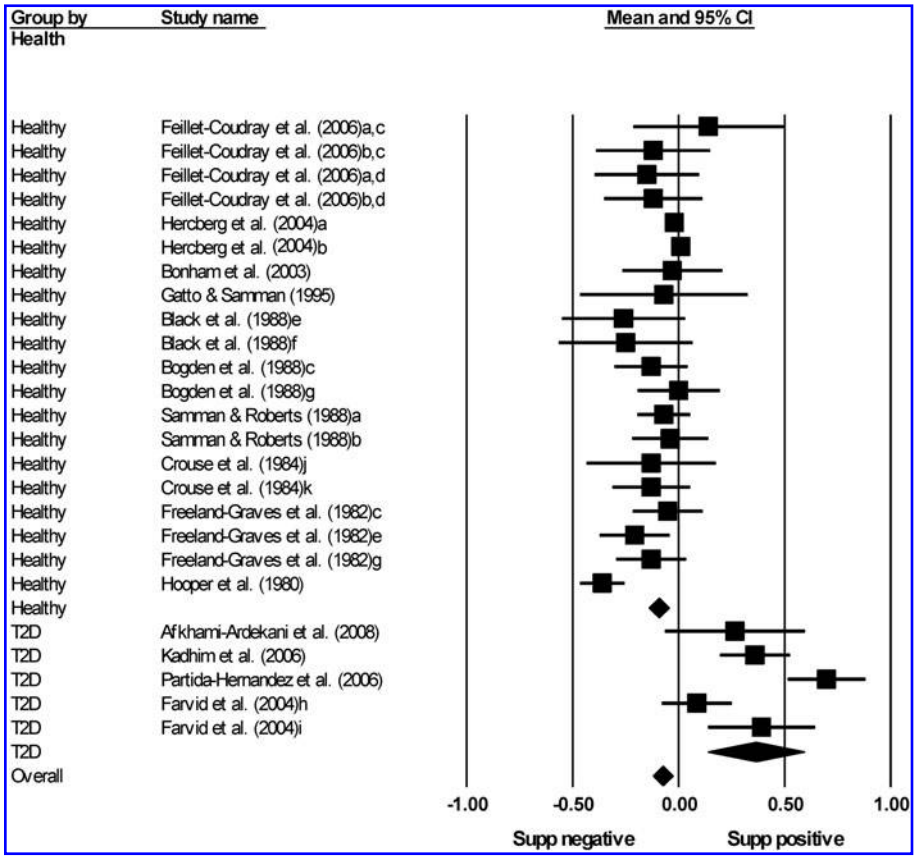

FIG. 3. Change in HDL cholesterol (mmol/L) by health status (2, 22, 24, 25, 44, 57, 60, 68, 71, 86, 90, 100, 155, 177). Data systematically extracted from controlled trials show no overall significant effect of zinc supplementation on plasma HDL cholesterol concentrations when evaluated using a random effects model of meta-analysis (67). Secondary analyses by health status revealed that zinc supplementation is associated with a significant decrease in plasma HDL cholesterol concentrations in individuals classified as healthy (-0.10 ± 0.02 mmol/L, $p < 0.001$; $n = 13{,}215$) and a significant increase in HDL cholesterol in those with type 2 diabetes mellitus ($+0.36 \pm 0.12$ mmol/L, $p < 0.01$; $n = 151$). a, male; b, female; c, 15 mg Zn/day; d, 30 mg Zn/day; e, 50 mg Zn/day; f, 75 mg Zn/day; g, 100 mg Zn/day; h, Zn + Mg; I, Zn Mg/vitC/vitE; j, trained; k, sedentary; HDL, high-density lipoprotein. ■, effect of individual studies; ◆, summary effect.

exploring the effect of zinc on CVD as part of a multinutrient regime. Populations already at risk of CVD appear more likely than healthy individuals to benefit from micronutrient supplementation. On the one hand, a large primary cardiovascular and cancer prevention study of healthy participants that incorporated 20 mg of zinc as part of its antioxidant supplementation protocol showed no effect of supplementation in the prevention of CVD (86) or on carotid atherosclerosis and arterial stiffness (236). Conversely, treatment of elderly chronic heart failure patients with multiple micronutrient supplements that were inclusive of zinc demonstrated improved left ventricular function and quality of life (224). In addition, obese Chinese women with hypertension and/or hyperglycemia and/or hyperlipidemia receiving multivitamin and mineral supplements, including 15 mg of zinc, demonstrated a reduction in blood pressure and serum C-reactive protein (214) (Table 2). In a randomized nutrition intervention trial in 29,584 participants selected from the general population of Linxian, China, treatment groups receiving a combination of zinc, vitamin A, riboflavin, and thiamine demonstrated a lowered stroke mortality for high-risk subjects (age $\geq$60 years, systolic blood pressure $\geq$160) but not for others (135). Further trials are needed to determine the effects of supplementing zinc alone in individuals exhibiting CVD risk factors and the nature of the interaction when zinc is found with other nutrients.

Diabetes mellitus

DM is a major risk factor for CVD, with 50% of diabetes-associated deaths being attributed to cardiovascular complications (26). Disturbances in zinc homeostasis and increased levels of oxidative stress each appear to play a major role in the pathogenesis of DM (52, 221). The involvement of oxidative processes in the disease is corroborated by the impact of proteins that protect against an accumulation of RS, such as MT, on the development of diabetic complications. Overexpression of MT in the pancreatic β-cells of streptozotocin-treated transgenic mice has been shown to reduce DNA damage and protect against hyperglycemia (34), while in humans a mutation in the *MT-1A* gene has been associated with the development of DM and its cardiovascular complications (72). Zinc deficiency associated with DM may impair the ability of zinc to induce MT expression and thereby increase the risk of oxidative stress-induced damage. Other critical pathways that are affected in DM and that are influenced by zinc and oxidative stress include those of insulin signaling, lipid metabolism, and immunity.

Zinc and Insulin. The characterizing feature of DM is the presence of chronic hyperglycemia, which is known to enhance oxidant production and impair antioxidant defense mechanisms (7, 106). Hyperglycemia is consequent upon the decreased secretion or action of insulin (98). Zinc has long been known to elicit insulin-like effects and it is this property that is perhaps most obviously affected by the alterations in zinc distribution and metabolism associated with DM. Under normal conditions zinc is abundant throughout the pancreas, but is particularly concentrated in the secretory vesicles of the β-cells, where it forms an integral component of the insulin crystalline structure (182), serving to stabilize the insulin granule by rendering it less soluble (208). Zinc has been shown to stimulate lipogenesis and inhibit lipolysis, attenuate hyperglycemia, and stimulate glucose transporter 4 translocation to the cell membrane. Recent evidence has also demonstrated a role for zinc in the induction of the PI3K/Akt cascade, which, in addition to its roles in regulating apoptosis and proliferation and promoting resistance to oxidative stress, is a major mediator of insulin signaling (Fig. 1). The serine/threonine kinase Akt directly targets and inactivates a number of the FoxO transcription factors, which are potent transactivators of genes involved in glucose metabolism, such as glucose-6-phosphatase, glucokinase, and phosphoenolpyruvate carboxykinase, and as such are important targets of insulin action (233). The ability of zinc to activate the PI3K/Akt signaling pathway is able to be achieved *via* attenuation of the activity of protein phosphatases, such as PTP1B (116) or possibly the Ser/Thr protein phosphatase 2A (PP2A) (120), which otherwise act to inhibit the PI3K-dependent activation of Akt (116).

When zinc homeostasis is altered, the insulinomimetic effects of zinc are likely to be impaired. Zinc depletion of the insulin molecule appears to leave insulin vulnerable to structural modification and, further, the oxidation of zinc-depleted insulin prior to its infusion into rats resulted in a lower biological activity compared to oxidized native insulin, suggesting that the zinc in insulin may function to protect it against oxidative damage (59). In addition, recent evidence suggests that single-nucleotide polymorphisms in the *ZnT8* gene are associated with impaired proinsulin conversion (109) and increased risk of developing type 2 DM (185). *ZnT8* belongs to the CDF/ZnT (SLC30) family of zinc transporters, which promotes zinc efflux from the cell or sequestration into intracellular organelles, and along with *ZnT5* it is abundantly expressed in the pancreas. It has been shown to colocalize with insulin in insulin-secreting INS-1 cells and human pancreatic islets and appears to be involved in both zinc accumulation and regulation of insulin secretion in β-cells (37). Although initially suggested to be exclusively a β-cell transporter, recently *ZnT8* expression was observed also in α-cells (78), intimating that polymorphisms in the *ZnT8* gene may affect both α- and β-cell function. Additionally, *ZnT8* mRNA has been found to be expressed in the peripheral blood mononuclear cells of some but not all healthy individuals (66, 149) (Fig. 4), but whether such interindividual variation in some way reflects type 2 DM risk has yet to be explored.

Perturbed zinc homeostasis in DM appears to lead to an overall state of zinc deficiency, evidenced in part by the state of hyperzincuria consistently reported to be present in the disease. It has also been suggested that oxidative stress-induced zinc release may result in a cellular zinc deficiency in insulin-responsive tissue (81). One of the possible effects of low zinc availability is a reduction in CuZnSOD levels. It has been proposed that CuZnSOD functions to protect insulin and β-cells from oxidative damage (194). Further, CuZnSOD protects NO from being quenched by excess $O_2^{\bullet -}$; NO has a role in promoting glucose uptake and supply in skeletal muscle and facilitates the binding of insulin to its receptor (159). On the other hand, even where an overall zinc-deficient state exists, it is plausible that the oxidative stress associated with DM may equally lead to localized increases in intracellular free zinc by stimulating the inappropriate release of zinc from zinc-binding proteins such as MT. Increased levels of RS

Table 2. Effect of Zinc Supplementation, Alone or in Combination with Other Nutrients, in Subjects with Cardiovascular Disease, Diabetes Mellitus, or Related Risk Factors

Reference	n (*treatment, control*)	*Participants (health, gender, age range, or mean)*	*Trial design and duration*	*Zn dose (mg/day, anion)*	*Other nutrients*	*Effect of Zn treatment on cardiovascular disease or DM-related outcome variables and plasma/serum [Zn]*
Wang *et al.* (214)	32, 32	Hypertension and/or hyperglycemia and/or hyperlipidemia, F, 42.0 ± 7.1	RCT, parallel, double blind, 26 weeks	7.5, anion not reported	Multiple	No change in SBP, DBP, serum C-reactive protein, serum [Zn] ↓ SBP, DBP compared to control
	32, 32	Hypertension and/or hyperglycemia and/or hyperlipidemia, F, 42.0 ± 7.1	RCT, parallel, double blind, 26 weeks	15, anion not reported	Multiple	↓ serum C-reactive protein compared to control and baseline ↑ serum [Zn] compared to control and baseline
de Sena *et al.* (50)	20, 17	Type 1 DM, M/F, 4.1–16.5	CT, parallel, 4 months	7.5–15, glycine	—	↑ HbA1c No change in plasma [Zn]
Witte *et al.* (224)	14, 14	Stable chronic heart failure, gender not reported, 75.4 ± 4.2	RCT, parallel, double blind, 39 weeks	15, anion not reported	Multiple	↑ LVEF, QoL No change in inflammatory cytokines Effects on plasma/serum [Zn] not reported
Parham *et al.* (153)	21, 18	Type 2 DM with microalbuminuria, M/F, 52.0 ± 9.3 (group 1) 54.5 ± 9.2 (group2)	RCT, crossover, double blind, 13 + 13 weeks	30, sulfate	—	↓ urinary albumin with Zn supplementation ↓ HbA1c in one group only No change in creatinine clearance, fasting plasma glucose, total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol ↑ serum [Zn]
Al Maroof and Al-Sharbatti (4)	50, 51	Type 2 DM, M/F, 54.6 ± 9.2	CT, parallel, single blind, 13 weeks	30, sulfate	—	↓ HbA1c No change in FBS ↑ serum [Zn]
Farvid *et al.* (57)	16, 18	Type 2 DM, M/F, 51.1 ± 7.5 (intervention) 49.6 ± 9.2 (control)	RCT, parallel, double blind, 13 weeks	30, sulfate	200 mg Mg	No change in total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol ↑ serum [Zn] compared to control and baseline
	17, 18	Type 2 DM, M/F, 50.6 ± 9.7 (intervention) 49.6 ± 9.2 (control)	RCT, parallel, double blind, 13 weeks	30, sulfate	200 mg Mg + 200 mg Vitamin C + 150 mg Vitamin E	No change in total cholesterol, triglycerides, LDL-cholesterol ↑ HDL-cholesterol compared to control and baseline ↑ serum [Zn] compared to control and baseline
Anderson *et al.* (5), Roussel *et al.* (173)	27, 29	Type 2 DM, M/F, 51.5 ± 1.6 (intervention) 55.5 ± 1.4 (control)	RCT, parallel, double blind, 26 weeks	30, gluconate	—	Decreasing trend in HbA1c No change in total cholesterol, HDL-cholesterol, erythrocyte CuZnSOD, GPx ↓ TBARS compared to baseline ↑ serum [Zn] compared to baseline

	27, 29	Type 2 DM, M/F, 53.8 ± 1.9 (intervention) 55.5 ± 1.4 (control)	RCT, parallel, double blind, 26 weeks	30, gluconate	400 μg Cr	Decreasing trend in HbA1c No change in total cholesterol, HDL-cholesterol, erythrocyte CuZnSOD, GPx ↓ TBARS compared to baseline ↑ serum [Zn] compared to baseline
Faure *et al.* (58)	10, 8	Type 1 DM with/without early retinopathy, M/F, 32.2 ± 8.1	CT, cross-over, 13 + 13 weeks	30, gluconate	—	↓ CuZnSOD in patients with retinopathy ↓ TBARS in both groups ↑ GPx in patients with retinopathy ↑ serum [Zn] in both groups
Kadhim *et al.* (100)	18, 15	Type 2 DM, M/F, 51.7 ± 7.1	CT, parallel, 13 weeks	50, acetate	10 mg melatonin	↓ Total cholesterol, triglycerides, LDL-cholesterol ↑ HDL-cholesterol ↓ microalbuminuria Effects on plasma/serum [Zn] not reported
Cunningham *et al.*, 1994 (46)	6, 7	Type 2 DM, M/F, 25 ± 3 (DM intervention) 32 ± 2 (non-DM intervention as control)	CT, parallel, 4 weeks	50, gluconate	—	↑ HbA1c in type 2 DM group at weeks 2 and 4 compared to baseline;↑ in HbA1c in nondiabetic supplemented group that stabilised at wk 2 No change in plasma [Zn] (but↑ urinary [Zn] in all subjects)
Partida-Hernandez *et al.* (155)	27, 27	Type 2 DM, M, 49.1 ± 6.0	CT, crossover, double blind, 12 + 12 weeks	100, sulfate	—	↓ Total cholesterol, triglycerides ↑ HDL-cholesterol No change in LDL-cholesterol, HbA1c ↑ serum [Zn] compared to baseline
Gupta *et al.*, 1998 (77)	15, 15	Type 2 DM with/without neuropathy, M/F, 49.95 ± 11.0	RCT, parallel, double blind, 6 weeks	150, sulfate	—	↑ FBS, PPBS, motor nerve conduction velocity ↑ Serum [Zn] compared to control and baseline
Afkhami-Ardekani *et al.* (2)	20, 20	Type 2 DM, M/F, 52.7 ± 8.6	RCT, parallel, 6 weeks	150, sulfate	—	↓ SBP, no change in DBP Decreasing trends in FBS, PPBS, HbA1c; ↓ HbA1c at follow-up (12 weeks) ↓ Total cholesterol, triglycerides, LDL-cholesterol Increasing trend in HDL-cholesterol Effects on plasma/serum [Zn] not reported
Niewoehner *et al.* (144)	9, 4	Type 2 DM and low serum Zn (<10.7 μmol/L), gender not reported, 61 ± 2	Design not reported, 6–8 weeks	150, sulfate	—	No change in HbA1c ↑ serum [Zn] compared to control and baseline

CT, controlled trial; DBP, diastolic blood pressure; DM, diabetes mellitus; FBS, fasting blood sugar; GPx, glutathione peroxidase; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; LVEF, left ventricular function; PPBS, postprandial blood sugar; QoL, quality of life; RCT, randomized controlled trial; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances.

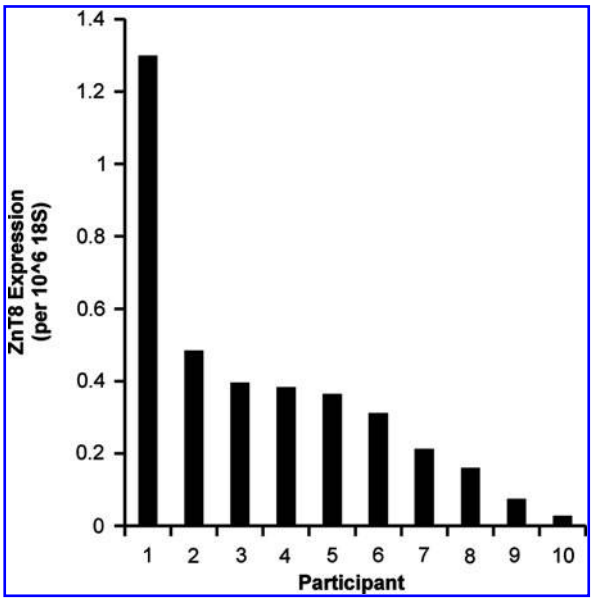

FIG. 4. ZnT8 mRNA expression in PBMCs of healthy individuals. ZnT8 mRNA expression, measured using TaqMan real-time PCR, was detected in the PBMCs of only 10 out of a total of 40 healthy individuals (66). The subjects (20 males and 20 females) donated fasting blood samples. Total RNA was isolated from PBMCs using the RNAqueous Small Scale Phenol-Free Total RNA Isolation Kit (Applied Biosystems) and its purity and yield were determined by UV spectrophotometry (Nanodrop/Thermo Scientific). RNA integrity was verified by ethidium bromide staining after 2% agarose gel electrophoresis. Total RNA was reverse transcribed into cDNA in two steps using the Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen). Relative quantification of ZnT8 mRNA was then conducted using TaqMan real-time PCR (ABI7500 Fast Sequence Detection System; Applied Biosystems), with amplification up to 40 cycles. Sample reactions were performed in triplicate with duplicate no-template controls. Messenger RNA levels were normalized to 18S rRNA expression as an endogenous reference and quantified using the ΔC_P method. PBMCs, peripheral blood mononuclear cells; ZnT, zinc transporter.

can result in the irreversible oxidation of MT, leading to degradation of the protein, probably *via* ubiquitination (42). Increases in intracellular free zinc may result in excessive stimulation of cellular signaling cascades with a range of potential pathophysiological consequences (116). In a similar vein, zinc released by β-cells under conditions of insulin hypersecretion appears to act as a paracrine effector of β-cell death (103).

Dyslipidemia. Type 2 DM and insulin resistance are associated with perturbed postprandial lipid metabolism, which itself is a risk factor for CVD. The major changes in lipid profile in type 2 DM are an increase in triglycerides, a reduction in HDL cholesterol, and the increased appearance of small, dense LDL particles, which are particularly susceptible to oxidative modification.

A range of studies in humans and animals suggest that zinc has the potential to affect triglyceride and lipoprotein metabolism and hence impact DM and CVD risk. While the mechanisms have yet to be established, they may again involve zinc's role in insulin action, which has been proposed as an independent predictor of plasma HDL and triglyceride concentrations (232). Moreover, as with glucose metabolism, many of the effects of insulin on lipid metabolism are mediated by the zinc and redox-responsive PI3K/Akt pathway and its regulation of FoxO-dependent gene transactivation (Fig. 1). The mechanism by which zinc influences the PI3K/Akt pathway in lipid metabolism appears to differ, however, from that of glucose metabolism. While in glucose-related signaling PTP is the key inhibitor targeted by zinc, the inactivation of lipid products appears instead to be modulated by the lipid phosphatase PTEN (116). The ability of zinc to inhibit PTEN has been demonstrated in epithelial cells, where zinc treatment resulted in both the downregulation of PTEN mRNA levels and a reduction in PTEN protein levels as a result of proteasomal degradation (225). The many roles ascribed to FoxO proteins in lipid metabolism that might conceivably be affected by changes to zinc homeostasis in DM include the stimulation of lipoprotein lipase expression, which is responsible for the breakdown of plasma triglycerides, the suppression of lipogenesis in the liver (233), and the transcriptional regulation of microsomal triglyceride transfer protein, which is involved in hepatic lipoprotein assembly (188). FoxO has further been suggested to induce the translocation of the fatty acid transport protein cluster of differentiation 36 to the plasma membrane where it can promote fatty acid uptake (16).

An interaction between zinc and leptin also has been proposed in type 2 DM. Among its many roles in lipid metabolism, leptin is critically involved in fatty acid oxidation and the regulation of intracellular triglyceride levels, the excessive accumulation of which can induce lipotoxicity in non-adipocytes and ultimately result in insulin resistance (33). Like insulin, leptin is able to invoke PI3K/Akt-dependent signaling (Fig. 1) and crosstalk has been observed between the two pathways (102). Most recently, leptin-deficient Akt knockout mice were shown to display hyperglycemia and reduced insulin levels, both of which were able to be normalized by the restoration of leptin despite the existence of β-cell dysfunction (35). As with insulin, PTP has been demonstrated to be a key inhibitor of the leptin PI3K signaling pathway (143), possibly intimating a role for zinc in leptin signaling *via* its ability to inactivate this enzyme. Moreover, a direct influence of zinc on leptin is suggested by the finding that zinc deficiency in humans is accompanied by a reduction in leptin concentrations (128), although this relationship may be complicated by such factors as baseline zinc status, oxidative stress levels, body weight, and gender. In postmenopausal women with type 2 DM, for example, while both obese and nonobese subjects demonstrated lower plasma zinc levels than the nonobese controls, only obese subjects demonstrated a difference in leptin levels, which were negatively correlated with plasma zinc (113). The effect of zinc status on the actions of leptin in oxidative stress-related conditions such as DM requires further investigation.

Immune Function. Both type 1 and type 2 DM exhibit an impaired immune function as part of their pathogenesis that ultimately results in a decreased functional β-cell mass; while type 1 DM is primarily an autoimmune disorder that leads to rapid β-cell destruction, the failure of β-cells in type 2 DM

occurs over a prolonged period and involves the chronic activation of the innate immune system (55). The sustained or aberrant expression in DM of a number of important immune mediators that are both zinc and RS responsive, including NF-κB and the proinflammatory cytokines IL-1β and IL-6, suggests a potential interaction between the impaired immunity and the perturbed cellular zinc and redox homeostases associated with the disease.

The wide involvement of zinc in the immune system is well established (117), with an initial consequence of zinc deficiency being an impairment of immunological functions (165). Zinc is crucial for the normal development and function of cells mediating both innate and acquired immunity (94). In its signaling capacity, available zinc has the ability to regulate both negatively and positively the secretion of inflammatory cytokines such as IL-1β and IL-6 by modulating, for example, the signaling pathways of IL-1R and toll-like receptor. Both of these pathways converge on a common IκB kinase complex that activates NF-κB by phosphorylating the NF-κB inhibitory protein IκB-α, suggesting that the dual effect of zinc in each case may reflect its ability to induce or inhibit NF-κB activation, which, as discussed previously, may be dependent in turn on the concentration of zinc under study or the particular cellular milieu.

Increased concentrations of IL-1β have been observed in the pancreatic islet in humans with type 2 DM (125) and human islets have been shown to respond to metabolic stress *in vitro* by increasing IL-6 release (56). A prospective examination of the effects of IL-1β, IL-6, and TNF-α on the development of type 2 DM found that participants with detectable levels of IL-1β and elevated levels of IL-6 in plasma had a threefold increased risk of developing DM compared to the reference group (189). Both IL-1β and IL-6 are pleiotropic and are known to exert both beneficial and detrimental effects on a variety of cell types, including the insulin-secreting β-cells of the pancreas, depending on the cytokine concentration and the duration of exposure. Higher doses and longer exposure times impair glucose-stimulated insulin secretion and, at least in the case of IL-1β, increase β-cell apoptosis (54).

β-cell destruction can be mediated by autoreactive T-lymphocytes such as $CD4^+$ and $CD8^+$ cells; cytokines have been shown to induce expression of the Fas (CD95, APO-1) receptor in the β-cell, thereby sensitizing it to T-lymphocyte-mediated destruction (55). The involvement of T-cells in β-cell failure, and in the inflammatory state more broadly, further implicates perturbations in zinc homeostasis in the pathogenesis of DM. Zinc deficiency is well known to disrupt the development and function of T-cells by causing, for instance, a reduction in thymic involution (218) and a decrease in the $CD4^+$ to $CD8^+$ cell ratio (165), both effects that have been shown to be corrected by zinc supplementation.

A putative role for nutritional zinc supplementation in DM

A recent systematic review and meta-analysis determined that there is insufficient evidence to support a role for zinc supplementation in the prevention of type 2 DM, with only one study meeting the inclusion criteria of the review (18). In contrast, a large prospective study recently concluded that higher zinc intakes may be associated with a slightly lower risk of type 2 DM in women (194). Moreover, upon examining the literature, there are indications that zinc may ameliorate oxidative-stress-related parameters in the established disease (Table 2). Zinc supplementation of individuals with type 2 DM with 30 mg Zn/day for 6 months resulted in decreased levels of thiobarbituric acid reactive substances (5). Similarly, in type 1 diabetics, daily supplementation of 30 mg zinc for 3 months reduced the level of thiobarbituric acid reactive substances and increased selenium glutathione peroxidase activity (58). Further, zinc has been shown to generate beneficial effects in diabetic neuropathy (77) and to reduce oxidative stress in type 1 diabetic patients with retinopathy (58). On the other hand, two studies detected an increase in hemoglobin A1c in type 1 diabetic subjects supplemented with zinc in the amounts of 7.5–15 mg/day (50) and 50 mg/day (46), indicating a further deterioration of metabolic control and increased oxidative stress associated with hyperglycemia. These results were not reflected in type 2 DM, however, with decreased hemoglobin A1c levels observed upon zinc supplementation of 30 mg/day in one study (4) and no significant changes detected in others (5, 144).

In a meta-analysis of randomized controlled trials investigating the effect of zinc supplementation on plasma lipids in humans, a significant increase in plasma HDL cholesterol was observed in subjects with type 2 DM (Fig. 3). Four studies (five interventions) contributed to this result (2, 57, 100, 155) (Table 2). In contrast, apparently healthy participants demonstrated a decrease in HDL (Fig. 3), highlighting the complexities of zinc supplementation in humans (67).

Future Directions

There are a number of positive indications for zinc supplementation in CVD and DM, raising the possibility of specific therapeutic manipulation by zinc-based treatments (32). The desirability of zinc supplementation needs to take account of the potential for adverse effects, however, such as the ability of zinc to abate the intestinal absorption of copper. As a consequence of reduced copper uptake, the activities of copper metalloenzymes are compromised, including CuZnSOD and EC-SOD (176), and in case reports, copper deficiency has been shown to increase the plasma cholesterol concentration (111) and reduce the clearance of glucose (110). *In vitro*, supplementation with high zinc doses is recognized to produce adverse consequences similar to those observed in zinc-deficient conditions, such as the inhibition of T-cell functions and aberrant expression of cytokines (94). Such results indicate the need for caution in the administration of zinc in the clinical setting and the desirability of conducting further well-designed randomized controlled trials to provide cogent insight into safe and desirable levels of zinc supplementation in varied populations and to determine the extent to which the results of *in vitro* and animal studies are relevant to human health. The appropriate design of zinc supplementation trials, however, and their ability to determine the efficacy of zinc in ameliorating oxidative stress parameters in particular, are not straightforward matters. Perhaps the most pervasive issue is the lack of a sensitive and specific biomarker of zinc status, making zinc supplementation studies difficult to compare; zinc-deficient subjects will likely react differently to zinc supplementation than zinc sufficient ones. Further investigation of the molecular mechanisms that underpin the transport, sensing,

and distribution of zinc is necessary also to explain the zinc effects in humans. In a similar vein, there is a need to develop methods of oxidative stress assessment that further clarify the role of RS in pathological conditions.

Recognition of the public health importance of zinc sufficiency continues to expand, as does knowledge of the multitude of biological pathways affected by zinc and its interaction with redox metabolism. Impaired zinc homeostasis and increased levels of oxidative stress feature prominently in a number of CVD and DM-related disorders, including atherosclerosis, hypertension, insulin resistance, and dyslipidemia. The interplay between zinc, redox signaling, and chronic disease warrants further examination.

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Abbreviations Used

CAT = catalase
CDF = cation diffusion facilitator
CuZnSOD = copper, zinc SOD
CVD = cardiovascular disease
DBP = diastolic blood pressure
DM = diabetes mellitus
EC-SOD = extracellular SOD
eNOS = endothelial nitric oxide synthase
ERK = extracellular signal-regulated kinase
FBS = fasting blood sugar
FoxO = forkhead box
G6Pase = glucose-6-phosphatase
GCL = glutamate cysteine ligase
GCLC = GCL catalytic subunit
GCLM = GCL modifier subunit
GK = glucokinase
GPx = glutathione peroxidase
GSH = glutathione
H_2O_2 = hydrogen peroxide
HbA1c = hemoglobin A1c
HDL = high-density lipoprotein
IκB = inhibitor of NF-κB
IL = interleukin
IL-R = interleukin receptor
iNOS = inducible NOS
INSR = insulin receptor
JNK = c-Jun N-terminal kinase
LEPR = leptin receptor
LPL = lipoprotein lipase
LVEF = left ventricular function
MAPK = mitogen activated protein kinase
MT = metallothionein
MTF-1 = metal responsive element-binding transcription factor-1
MTP = microsomal triglyceride transfer protein
NADPH = nicotinamide adenine dinucleotide phosphate
NF-κB = nuclear factor-kappa B
NO = nitric oxide
Nrf2 = nuclear redox factor 2
$O_2^{\bullet -}$ = superoxide
oxLDL = oxidized low-density lipoprotein
PBMCs = peripheral blood mononuclear cells
PDK = phosphoinositide-dependent protein kinase
PEPCK = phosphoenolpyruvate carboxykinase
PI3K = phosphoinositide 3′-kinase
PIP3 = phosphatidylinositol 3,4,5-trisphosphate
PKC = protein kinase C
PPBS = postprandial blood sugar
PTEN = phosphatase and tensin homolog
PTP = protein tyrosine phosphatase
QoL = quality of life
RS = reactive species
SBP = systolic blood pressure
SOD = superoxide dismutase
TBARS = thiobarbituric acid reactive substances
TNF = tumor necrosis factor
TPEN = *N,N,N′,N′*-tetrakis-(2-pyridylmethyl)-Ethylenediamine
Zip = Zrt- and Irt-like protein
ZnT = zinc transporter

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