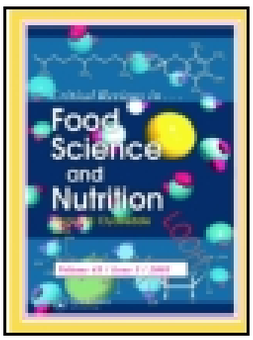

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REVIEW



# Diabetes and zinc dyshomeostasis: Can zinc supplementation mitigate diabetic complications?

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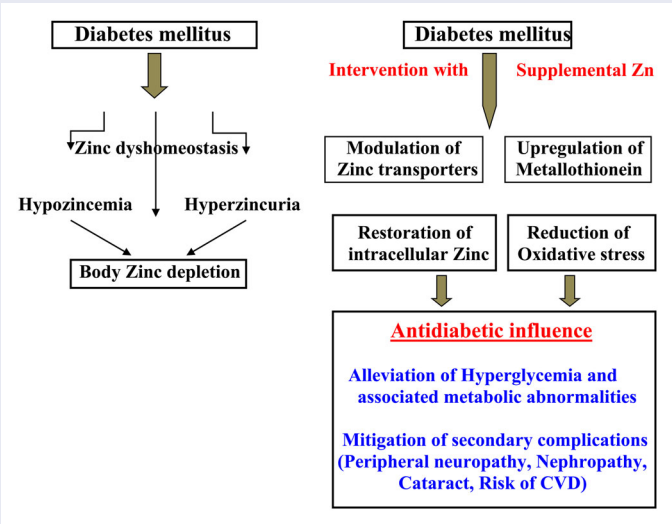
**ABSTRACT**

Zinc present in the islet cells of the pancreas is crucial for the synthesis, storage, and secretion of insulin. The excretion of large amounts of zinc from the body is reported in diabetic situations. Zinc depletion and increased oxidative stress have a major impact on the pathogenesis of diabetic complications. It would be most relevant to ascertain if intervention with supplemental zinc compensating for its depletion would beneficially mitigate hyperglycemia and the attendant metabolic abnormalities, and secondary complications in diabetes. An exhaustive literature search on this issue indicates: (1) Concurrent hypozincemia and decreased tissue zinc stores in diabetes as a result of its increased urinary excretion and/or decreased intestinal absorption, (2) Several recent experimental studies have documented that supplemental zinc has a potential hypoglycemic effect in the diabetic situation, and also beneficially modulate the attendant metabolic abnormalities and compromised antioxidant status, and (3) Supplemental zinc also alleviates renal lesions, cataract and the risk of cardiovascular disease accompanying diabetes mellitus, and help restore gastrointestinal health in experimental diabetes. These studies have also attempted to identify the precise mechanisms responsible for zinc-mediated beneficial effects in diabetic situation. The evidence discussed in this review highlights that supplemental zinc may significantly contribute to its clinical application in the management of diabetic hyperglycemia and related metabolic abnormalities, and in the alleviation of secondary complications resulting from diabetic oxidative stress.

**KEYWORDS**

Diabetes mellitus; oxidative stress; secondary complications; zinc dyshomeostasis; zinc supplementation

**GRAPHICAL ABSTRACT**




## Introduction

Hyperglycemia manifested in conditions of diabetes mellitus is the result of defects in insulin secretion from islet cells of the pancreas or insulin sensitivity or both. Uncompromised diabetes if prolonged leads to damage, dysfunction, and failure of several organs, particularly the nerves, kidneys, eyes, blood vessels, and heart. $\beta$–cell destruction causes type 1 diabetes mellitus, usually leading to absolute insulin deficiency. The majority of type 1 or juvenile-onset diabetes is predominantly due to pancreatic islet $\beta$–cell destruction attributable to an autoimmune development. Partial insulin

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deficiency along with insulin resistance or a defect in insulin secretion with/without insulin resistance leads to type 2 diabetes, which is a chronic and multifactorial disease characterized by hyperglycemia and glucose intolerance.

According to the International Diabetes Federation, the estimated number of adult diabetics (aged between 20 and 99 years) worldwide in 2017 is around 451 million, with type 2 amounting to about 90% of the cases. This amounts to 8.5% of the population distributed equally among both women and men (Cho et al. 2018). The worldwide prevalence of diabetes is estimated to become 690 million by 2045. India and China have a higher number of diabetics than any other country (62 million in India accounting for 7.1% of India's adult population) (Hu 2011), this high prevalence having been accredited to a combination of genetic predisposition and switching over to a high-calorie diet and low-physical activity lifestyle.

In the management of any disease condition, dietary elements play a very significant role. The role of food components in the etiology of chronic syndromes such as diabetes and their management has been gradually understood in recent decades. Before the introduction of the therapeutic practice of insulin, the diet was the main form of management of the disease. In type-1 diabetes involving children and adolescents, the real dietary challenge is to maintain good glycemic control without compromising adequate energy to ensure growth and development. On the other hand, in type-2 diabetes involving adults, the dietary challenge is to achieve a 5–10% reduction in body weight, and maintain good glycemic control and reduce cardiovascular risk factors (Barclay et al. 2010). Optimal dietary strategy in diabetes includes a diet with reduced saturated fat and sodium, moderate protein, and high dietary fiber and low glycaemic index carbohydrates. Consumption of liberal amounts of fresh vegetables and fruits is recommended in place of high-calorie foods. Staple food should be drawn from whole/unrefined food grains (cereals) high in fiber as a healthy source of carbohydrate. Legumes (beans and lentils)/pulses are recommended to be a low-fat starchy source of protein and fiber.

There is an intricate relationship between the micronutrient zinc (Zn) and insulin hexamer structure, and that zinc deficiency has been recognized during diabetes. Incidentally, the highest amount of zinc within the human body is present in the pancreatic $\beta$ cells. Zn concentrated in islet cells of the pancreas is concerned with the synthesis, storage, and secretion of insulin from this endocrine organ (Li 2014). Excessive loss of Zn from the body via urinary and fecal excretion is reported in diabetic situations consequently causing a decrease in tissue Zn stores (Isbir et al. 1994). While it is established that excretion of Zn from the body is markedly increased in diabetic patients, oral Zn supplementation is expected to provide sufficient benefit and a protective role for Zn nutrition in this condition (Barman and Srinivasan 2016). Since the body's Zn depletion and increased oxidative stress have a major impact on the pathogenesis of diabetic complications, it would be most relevant to ascertain if intervention with supplemental zinc compensating for its depletion would beneficially mitigate hyperglycemia and the attendant metabolic abnormalities, and secondary complications in diabetes. The present review highlights the currently available information on this issue compiled from an exhaustive literature search.

## Zinc as a micronutrient mineral

Micronutrient Zn is essential for numerous aspects of cellular metabolism. Everyday intake of Zn is essential to sustain its steady-state since the body lacks a dedicated Zn storage system (Rink and Gabriel 2000). Zn is an obligatory metallic cofactor for many proteins that participate in cell proliferation, differentiation, apoptosis (Jansen, Karges, and Rink 2009), and gene expression (Devirgiliis et al. 2007). Zn is also known for its pro-antioxidant role in the cell as it protects sulfhydryl groups from oxidative damage and subsequently inhibits the production of reactive oxygen species (Prasad and Bao 2019). Zn also has anti-inflammatory potential as it acts as negative feedback in the inflammatory pathway (Cruz, de Oliveira, and Marreiro 2015). Zn is understood to stimulate the expression of metallothionein protein which is a potent antioxidant and exalted expression of the same may be reasonably protective in the diabetic situations (Tachibana et al. 2014). Zn is also considered a vital micronutrient required for normal growth and development during pregnancy, childhood, and adolescence (Wolfgang and Sandstead 2006).

## Zinc transporters

Normally, dietary Zn is absorbed to an extent of 33% by duodenal and jejunal enterocytes (Fosmire 1990). A large portion of whole body Zn is stored in skeletal muscle (60%), bones (30%), and to a lesser extent in liver and skin (5%). The rest of Zn in the body is distributed in the brain, mammary gland, pancreas, and kidneys. Only 0.1% of total body Zn is found in the blood in association with albumin and $\alpha$2-macroglobulin. Compared to biliary secretion and subsequent elimination through feces, loss of Zn through urine is negligible. Zn distribution in the body is well regulated at cellular and systemic levels through harmonized regulation, influx, and efflux system, where Zn transporters play an important role. Intracellular Zn homeostasis is intricately controlled. This is achieved through precise regulation of compartmentalization and the presence of transmembrane Zn transporter proteins, belonging to the ZnT family for Zn efflux/sequestration and ZIP family for zinc influx (Kambe 2011). How the expression of these transporters is regulated to effectively facilitate Zn flux, and hence intracellular Zn metabolism is less understood.

Synchronized zinc mobilization is ensured by Zn transporters belonging to the Zrt-, Irt-like protein (ZIP) family, and the Zn transporter (ZnT) family. ZIP and ZnT transporters are pivotal for sustaining Zn homeostasis. Thus, it plays critical physiological functions and intensely affects human health. The study of ZIP and ZnT transporter proteins is, therefore, receiving much concern and attention.

Ten homologous Zn transporter proteins (ZnT-1 to ZnT-10) have been described in mammalian cells (Seve et al. 2004). These proteins belong to the Cation Diffusion Facilitator family. ZnT-1 and ZnT-2 are located either in the plasma membrane or in lysosomal vesicles, respectively; these confer Zn resistance by facilitating Zn efflux from the cytoplasm (Palmiter, Cole, and Findley 1996a). ZnT-3 and ZnT-4 are localized in intracellular vesicles and these transporters are dedicated to Zn secretion pathways (Murgia et al. 1999). ZnT-3 is tissue-specific, and found mainly in the brain (Wenzel et al. 1997) and testis (Palmiter et al. 1996b). ZnT-4 is largely found in the brain and epithelial cells. This transporter is essential in mammary epithelia for regulating Zn content in milk (Huang and Gitschier 1997) and is known to have a role in mast cell Zn homeostasis (Ho et al. 2004). ZnT-5 is expressed more in the pancreas, liver, kidney, muscle, and heart, and is mainly localized in the Golgi apparatus (Kambe et al. 2002). ZnT-5 plays an important role in the cells that deal with the cardiac conduction system (Inoue et al. 2002). ZnT-6 and ZnT-7 are expressed in various tissues and localized in the Golgi apparatus (Kirschke and Huang 2003). ZnT-5 and ZnT-7 are required for the activation of glycosyl phosphatidylinositol anchored Zn -dependent enzymes (Suzuki et al. 2005). The essential role of $Zn^{2+}$ ions in pancreatic islet cells in the processing and storage of insulin and its precise regulation has been recently reviewed (Chabosseau and Rutter 2016). ZnT-8 which is a highly $\beta$-cell-specific Zn transporter has received the most attention because of its association in the etiology of both type-1 and type-2 diabetes (Chimienti, Favier, and Seve 2005). ZnT-8 is also shown to facilitate the accumulation of Zn from the cytoplasm into intracellular vesicles in the $\beta$-cell of the pancreas and providing Zn for the formation of Zn-insulin crystal.

Mutations in ZIP and ZnT transporter genes have been recognized in some congenital human diseases. Moreover, deregulation of the expression and activity of both transporters has been proposed in the pathogenesis of cancer, immunological impairment, diabetes mellitus, and neurodegenerative diseases, though comprehensive understanding is still elusive (Kambe 2011). The connotation of the expression of the two Zn transporter gene families — ZIPs and ZnTs and metallothionein (MT) with diabetes mellitus and with insulin secretion observed in clinical studies suggest a novel target, namely directly modulating zinc homeostasis in different organs to alleviate the deleterious complications of diabetes (Myers, Nield, and Myers 2012). Among the Zn transporters, ZIPs enable increase of intracellular zinc concentration by facilitating Zn uptake into the cytosol of the cell from the extracellular space, while ZnTs facilitate in the efflux of Zn from the cell sap. A general or tissue-specific upregulation of metallothionein is documented to protect from diabetes-induced cardiomyopathy and nephropathy and also to defend pancreatic beta cells from oxidative damage (Islam and Loots 2007).

## Zinc and insulin

A strong association between the micronutrient Zn, pancreatic function, and diabetes has been predicted for a long time (Chausmer 1998). The intricate relationship between Zn and insulin has been well recognized. Even before evidencing the association between Zn and insulin, it was understood that the addition of Zn would amend the duration of the effect of insulin (Chausmer 1998). After the introduction of insulin for commercial use in the 1930s, in vitro supplementation of Zn was also introduced to make protamine Zn insulin and Lente crystalline insulin; these products extended the duration of insulin action by delaying its absorption after the subcutaneous injection, and hence only fewer insulin injections are needed (Chausmer 1998).

Insulin is secreted by the pancreatic $\beta$-cells as a single chain peptide that is interlinked by two disulfide bonds. This proinsulin is sliced by the removal of a chain fragment 'C-peptide' to form an insulin monomer consisting of two ($\alpha$- and $\beta$-) peptide chains consisting of 51 amino acids, and cross-linked with each other by disulfide bonds (Weiss, Steiner, and Philipson 2000). Within the cell in the presence of Zn, these insulin monomers assume a dimeric form for storage purposes and subsequent discharging as a zinc crystal. Thus, zinc present in the islet cells is very much associated with the synthesis, storage, and secretion of the hormone insulin (Li 2014). In presence of Zn in vitro, the dimeric insulin further amasses into a hexamer form comprising of three dimeric units. This hexameric insulin form is relatively more stable. This hexameric crystalline form of insulin is regularly used for pharmacological applications. In vitro evidence suggests that with co-administration of Zn, insulin binds to isolated liver membranes to a better extent with lesser degradation (Arquilla et al. 1978). It is also advocated that antigenic determining factors are reformed by the exclusion of Zn from insulin by altering the molecule's conformation. Zn-free insulin was immunologically lesser active than Zn-insulin in immune hemolysis inhibition assays suggesting that there are numerous binding and stimulation mechanisms (Arquilla et al. 1978). Zn plays a significant protective role against the consequences of immune-mediated free radicals in the pancreatic islet cells, while the intricate mechanism of action is yet to be elucidated (Prasad and Bao 2019).

Therefore pancreatic beta cells certainly need very proficient and specific transporters to accrue ample amounts of Zn in secretion vesicles. ZnT-8 which belongs to the family of Cation Diffusion Facilitators possesses only pancreas-specific expression and this transporter is confined in secretory vesicles membrane and accelerates the accretion of Zn from the cytoplasm into the intracellular insulin-containing vesicles for supplying Zn for insulin maturation and storage processes (Chimienti, Favier, and Seve 2005; Kawasaki 2012). Hence the Zn transporter protein ZnT-8 is extremely protected during evolution, signifying the dominant role for Zn transportation into the pancreatic $\beta$-cells.

Zn exerts an insulin-like (insulin-mimetic) effect by particularly targeting the protein tyrosine phosphatase 1B (PTP 1B), which regulates the phosphorylation state of the insulin receptor (Haase and Maret 2005). Modulation of insulin signaling by zinc suggests a physiological role of Zn in insulin signal transduction. In diabetic conditions, oxidative stress

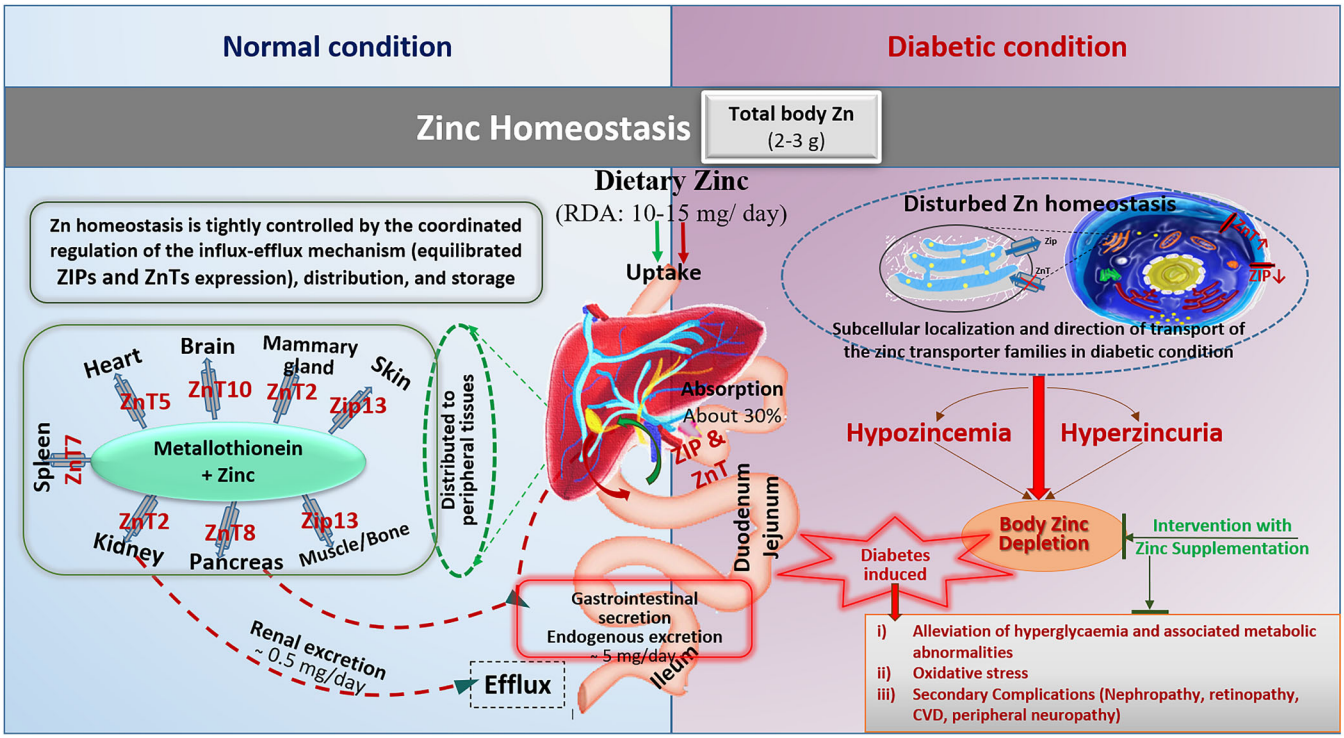


**Figure 1.** Zinc dyshomeostasis in diabetic condition.

as well as changes in Zn metabolism modifies the cell's sensitivity to insulin. Zn deficiency activates stress pathways possibly resulting in a loss of tyrosine phosphatase control, leading to insulin resistance (Haase and Maret 2005).

## Effects of diabetes on zinc metabolism

The characteristic impact of diabetes on Zn homeostasis in the body includes hypozincemia, hyperzincuria, and compromised gastrointestinal absorption of dietary Zn (Chausmer 1998) (Figure 1). Hypozincemia is a consequence of hyperzincuria or reduced gastrointestinal absorption of this micronutrient or could be both. While the normal serum Zn levels are 80 to 120 micrograms/dL, diabetic patients are reported to exhibit a marked decrease in plasma Zn and hyperzincuria suggesting that hyperglycemic condition probably hinders dietary Zn absorption (Luo et al. 2015; Fernández-Cao et al. 2018). A 20% decrease in serum Zn in Type-1 diabetes was observed to be the outcome of hyperzincuria (Isbir et al. 1994). Absolute excretions were prominent in diabetics and a positive association among Zn excretion and hemoglobin-A1c concentrations is documented (El-Yazigi, Hannan, and Raines 1993). However, it is not clear whether the concomitant hypozincemia and a reduction in tissue Zn stores in the diabetic situation is a consequence of hyperzincuria or an independent effect of hyperglycemia on the leaching of Zn stores from the tissues resulting in a subsequent loss of Zn from circulation.

In streptozotocin-induced experimental diabetic rats, excessive Zn excretion from the body has been regularly witnessed. Administration of insulin although reduces the extent of hyperglycemia, does not seem to noticeably amend the hyperzincuria accompanying this condition (Lau and Failla 1984). It is hypothesized that hyperglycemia affects the active transport of Zn by disturbing the equilibrium between the influx and efflux mechanisms involved in regulating the body Zn store. A down-regulation of fractional zinc transport could be linked to an increased expression of metallothionein in diabetic patients. Metallothioneins are a family of low molecular weight proteins localized to the membrane of the Golgi apparatus and these act as a carrier of metal ions including Zn and tend to be high wherever a higher concentration of metal ions is present. Interestingly, tissue Zn depletion occurs only in tissues that are dependent on insulin for glucose metabolism as there were no changes in bone Zn (Raz, Adler, and Havivi 1988). Data are inconsistent to signify connections with modifications in other trace elements, distinctive effects of hyperinsulinemia or hyperglycemia. Hyperglycemia and consequent glucose load in the kidney are responsible for hyperzincuria, as indicated by a significant correlation between the hemoglobin A1c and urinary Zn excretion in humans. HbA1c is a consistent, quantifiable indicator of chronic hyperglycemia. Further study has advocated that there is correspondingly compromised Zn absorption associated with hyperglycemia or diabetes (Kinlaw et al. 1983). Serum and urinary Zn levels in diabetic patients revealed no differences in diabetics, while hyperzincuria was evinced in these subjects (Golik et al. 1993).

## Effects of zinc deficiency on diabetes mellitus

Many studies have demonstrated the involvement of Zn deficiency in the pathogenesis of diabetes; however, the underlying molecular mechanisms in the role of Zn in regulating glucose metabolism remain unclear (Fukunaka and

Fujitani 2018). It is proposed that the inadequate distribution of Zn affects the onset of diabetes, and altered functioning of Zn transporter ZnT8 is now understood to be involved in the pathogenesis of type 2 diabetes (Fukunaka and Fujitani 2018). Thus, precise control of Zn homeostasis is critical for the prevention of diabetes.

In a study that evaluated the link between Zn-nutritional status and glycemic control and insulinemic markers in 82 type 2 diabetic patients, there was a strong association between reduced Zn status (in plasma and erythrocytes as well as a high urinary excretion) and poor glycemic control (indicated by higher glycated hemoglobin) in these patients (Bandeira et al. 2017). In a recent clinical trial involving around 250 type-2 diabetic patients, it has been reported that diabetics have Zn deficiency as compared to normal subjects and that poor glycemic control is invariably associated with diminished Zn levels in these patients (Farooq et al. 2020).

Diet prompted Zn deficiency in rats is shown to result in a decreased ability of the pancreas to release insulin in response to a glucose load (Quarterman, Mills, and Humphries 1966). Another study has observed a decline in glucose tolerance without altering insulin secretion in response to a glucose load in Zn-deficient hamsters (Boquist 1968). This suggests that Zn hinders the post-insulin receptor intracellular actions to result in a declined glucose tolerance, and causes decreased insulin secretion since high glucose is expected to prompt more exuberant insulin secretion. This indorses that Zn deficiency also diminishes the efficacy of the pancreas to respond applicably. Degraded granulation of the islet cell in zinc deficiency has also been documented (Boquist 1968). Since Zn is utilized for the storage and granulation of insulin within the $\beta$-cell, increased insulin secretion is associated with a reduction in $\beta$-cell Zn concentration; this evidence concurs with reduced islet cell insulin content in Zn-deficient states (Engelbart and Kief 1970). Although Zn deficiency has been associated with insulin resistance and conditions of hyperzincuria and low plasma Zn concentrations are attendant in diabetes, the potential effects of Zn supplementation in type-2 diabetes have been poorly studied. An animal study has compared the effects of dietary Zn deficiency (3 ppm Zn) and Zn supplementation (300 ppm Zn) for six weeks on glycemic control in db/db mice (Simon and Taylor 2001). Dietary supplementation of Zn attenuated hyperglycemia and hypoinsulinemia in db/db mice, thus suggesting the role of Zn in pancreatic function and glucose uptake in peripheral tissues.

Type-1 diabetes results from an autoimmune outbreak in the $\beta$-cell followed by the destruction of the cell. Cytokines are the intermediaries of the immune response, and they mediate cell damage in the autoimmune injury on the human $\beta$-cell in type-1 diabetes by generating oxygen free radicals, aldehyde formation, and lipid peroxidation in the islet cells. The association between cytokine-induced (Interleukin-1$\beta$, interferon-$\gamma$, and tumor necrosis factor-$\alpha$) pancreatic $\beta$-cell-damage and the accumulation of the end products of lipid peroxidation and nitrite (the end product of nitric oxide) has been well understood (Rabinovitch et al. 1996). It is also proposed that the Zn-metallothionein complex in the islet cell delivers protection against free radicals production (Chen et al. 2001). This suggests a role for Zn deficiency to prompt the progression of type-1 diabetes. The lipid peroxidation was allayed by pretreatment with Zn by upregulating the production of Metallothionein (Pauwels et al. 1994). Thus, there is a role of Zn in the $\beta$-cell to combat against the immune-intermediated free radical attack. Additionally, intracellular free radicals — superoxide radicals ($O^{2-}$), alkoxyl ($RO^{-}$), peroxyl radicals ($ROO^{-}$), lipid peroxides (LOOH), and hydrogen peroxide ($H_2O_2$) damage cells in the tissues concomitant with diabetes. Since Zn is a participating cofactor in antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, any variations of Zn metabolism like the non-availability of adequate Zn for these enzymes will contribute to the tissue damage in diabetes. In type-2 diabetes, there is an increased secretion of insulin in the early stage. As Zn is discharged from the $\beta$-cell along with insulin, a higher secretion of insulin causes increased cellular expulsion of Zn (Li 2014). The pancreatic islet cell can produce more insulin, but not Zn, and also through hyperzincuria in diabetes, co-secreted Zn is rather excreted. With the gradual loss of intracellular Zn and the lesser insulin secretion, the islet cell becomes more susceptible to injury. This is how Zn deficiency may stimulate the progression of type-2 diabetes.

## Beneficial effects of zinc supplementation on diabetes mellitus – insulin resistance, glycemic control, and oxidative stress

While Zn has a major role in the synthesis, storage, and secretion of insulin in physiological conditions as well as in diabetes mellitus, insulin resistance is associated with glucose intolerance. Zn is believed to contribute to insulin action by stimulating tyrosine kinase activity of insulin receptors (Ezaki 1989). Insulin resistance is fundamental to the etiology of type-2 diabetes. Hence, trials are warranted with zinc supplementation that measures the possible decrease of the insulin resistance and the eventual complications in type-2 diabetes mellitus.

A meta-analysis of more than 25 human studies has indicated the beneficial effects of Zn supplementation in diabetic patients (Jayawardena et al. 2012; Capdor et al. 2013). A majority of these trials have demonstrate that Zn supplementation has beneficial effects on glycemic control and a healthy lipid profile. A significant reduction in FBG and a decreasing tendency in HbA1c were consistently observed after Zn supplementation which was associated with an increased plasma Zn concentration. These meta-analyses strongly suggest that dietary Zn intervention may significantly contribute to the management of hyperglycemia in diabetes.

A recent review critically discusses the available information on the role of Zn in type 2 diabetes as well as metabolic syndrome and also speculates the mechanistic aspects of the potential beneficial effects of Zn (Ruz et al. 2019). Zn is not only involved in insulin secretion and its action in peripheral tissues, but Zn through its insulin-mimetic properties also promotes the insulin signaling pathway. It also has a role in the regulation of lipolysis. Zn plays a role in redox

metabolism through its antioxidant influence. Zn has been shown to have positive responses in terms of glucose control outcomes, at least among type 2 diabetic patients with Zn deficiency. While a good number of investigations have indicated the association between low zinc status and diabetes, information on the beneficial outcome of zinc supplementation on glycemic control in diabetes is inconclusive (Wang et al. 2019). A meta-analysis of all the randomized controlled trials suggests that Zn supplementation significantly reduces fasting blood glucose in diabetic subjects, thus supporting the notion that supplemental Zn has clinical potential as an adjunct therapy for managing diabetes mellitus.

Zinc supplementation in type-2 diabetics (oral 30 mg zinc sulfate/day for three months) produced beneficial effects in terms of elevating their serum Zn level and improving their glycemic control as indicated by lowered HbA1c concentration (Al-Maroof and Al-Sharbatti 2006). The effects of combined Zn and chromium (Cr) supplementation for six months (30 mg Zn/day as zinc gluconate or 400 mg Cr/day as Cr pidolate or combined Zn/Cr supplementation) on oxidative stress and glucose homeostasis has been reported in people with type-2 diabetes (Anderson et al. 2001). The data suggested the potential antioxidant effects of the individual and combined supplementation of Zn and Cr in type-2 diabetic patients as indicated by decreases of plasma TBARS. Supplementation, however, did not modify HbA1c nor glucose homeostasis significantly. Zn supplementation (oral zinc sulfate: 22 mg/day for four months) improved glycemic control as indicated by HbA1c% and fasting and postprandial glucose in adult diabetics (Gunasekara et al. 2011). Besides, Zn supplementation also lowered serum cholesterol and total cholesterol/HDL-cholesterol ratio while elevating their serum Zn level. Supplementation of a combination of zinc (10 mg per day) and vitamin A (12,500 IU per day) for twelve weeks could improve serum apoprotein A-I and apoprotein B, without any effect on fasting blood sugar and insulin in young patients with type-1 diabetes mellitus (Shidfar et al. 2010).

### Mitigation of diabetes-induced zinc dyshomeostasis by its dietary supplementation

The depletion of body zinc during diabetes calls for a need for Zn nutrition in this situation. A recent study has evidenced the mitigation of zinc dyshomeostasis by supplementation of this mineral in experimental diabetic rats, and this was mediated by regulating the expression of Zn transporters and metallothionein (Barman, Pradeep, and Srinivasan 2017). Groups of streptozotocin-induced hyperglycemic rats were treated with supplemental Zn (5 or 10-times of normal level) for six weeks. These animals were evaluated for intracellular Zn concentrations in different body tissues, and the expression of Zn transporters and metallothionein in specific vital tissues. Zn concentration depleted in different organs was significantly restored by Zn supplementation in diabetic animals. This study documented that depletion of zinc from body tissues and bones concomitant with its excessive excretion were moderated by Zn intervention in diabetic animals.

This study also evidenced that Zn ions cross biological membranes assisted by membrane-bound zinc transporter proteins — ZIPs (responsible for the influx) and ZnTs (responsible for the efflux and intracellular traffic) (Barman, Pradeep, and Srinivasan 2017). Up-regulated expression of Zn transporter proteins mediating efflux and influx of this mineral in a diabetic situation were beneficially modulated consequent to exogenous Zn treatment. In the diabetic situation, where the Zn efflux is concomitant with upregulated ZnTs (to meet the efflux-created zinc deficiency, the influx of Zn (mediated by ZIPs) was also higher in parallel. Supplemental Zn intervention to diabetic animals moderated the efflux of this mineral by down-regulating the expression of ZnTs, while ZIPs (responsible for the influx) were up-regulated to compensate for the loss of body Zn by the diabetes-induced efflux. Supplemental Zn also increased metallothionein expression in tissues to mitigate the oxidative stress in diabetes (Barman, Pradeep, and Srinivasan 2017). A protective role of metallothioneins induced by supplemental Zn intervention advocates that over-expression of this protein in diabetes could attenuate diabetic hyperglycemia and the concomitant oxidative stress. Thus, Zn supplementation has a promising beneficial effect in regulating Zn fluxes during the diabetic situation, exerted through modulation of the expression of Zn transporter proteins and metallothionein. This study has thus documented for the first time how Zn supplementation has a counteractive role on the diabetes-induced zinc-deprivation through regulating the expression of Zn transporters in a tissue-specific manner.

### Alleviation of hyperglycemia and associated metabolic abnormalities in diabetes by dietary zinc supplementation

Several studies have shown that Zn supplementation improves glucose handling in diabetic patients (Khan et al. 2013). A recent placebo-controlled study investigated if supplemental Zn-induced improvement in glucose handling could effectively prevent the progression of pre-diabetes to diabetes (Islam et al. 2016) by monitoring fasting blood glucose, HOMA parameters (beta-cell function, insulin sensitivity, and insulin resistance) at the end of 6 months of Zn supplementation (30 mg zinc sulfate per day). Six months of Zn intervention significantly improved FBG concentration and also improved Beta-cell function, insulin sensitivity, and insulin resistance. A recent placebo-controlled clinical trial carried for 12-months on 200 subjects to evaluated the beneficial effect of supplemental zinc (20 mg/day) on glycemic control and disease progression in prediabetes, development into type 2 diabetes was significantly lesser in Zn treated group (Ranasinghe et al. 2018). Fasting blood glucose, postprandial glucose, HOMA-IR, and hypercholesterolemia were lower in the supplemental Zn treated group along with a parallel improvement in $\beta$-cell function.

An animal study has documented the alleviation of hyperglycemia, hypoinsulinemia, and associated metabolic abnormalities in streptozotocin-induced diabetic rats by Zn supplementation (Barman and Srinivasan 2016). While

supplemental Zn (5-times and 10-times normal level) fed diabetic animals portrayed a significant control on hyperglycemia and insulin secretion, there was also a significant reduction in glucosuria and urinary excretion of proteins and urea. Exogenous Zn-fed diabetic rats showed a significantly higher plasma albumin content and lower plasma urea and creatinine levels. Significant alterations in insulin sensitivity indices– HOMA-IR, HOMA-B, and QUICKI were also evidenced in Zn-fed diabetic rats. Simultaneously, dietary Zn intervention significantly alleviated the pathological abnormalities in pancreatic islets of diabetic animals (Barman and Srinivasan 2016). This study has evidenced that dietary Zn intervention would bring about a partial amelioration of the severity of diabetic hyperglycemia and its associated metabolic abnormalities, insulin resistance, and altered pancreatic morphology.

### *Attenuation of diabetes-induced oxidative stress by dietary zinc supplementation*

Oxidative stress manifested by increased production of reactive oxygen species and/or reduced inherent antioxidant defense system plays a precipitative role in the pathogenesis of diabetes mellitus which further exacerbates lesions of multiple organs, particularly the eye lens, heart, and kidney. Zn plays an important role in the body's antioxidant defense in diabetic individuals by acting as a cofactor of SOD, by modulating the glutathione metabolism and by promoting metallothionein expression (Cruz, de Oliveira, and Marreiro 2015). This apart, Zn also ameliorates oxidative stress in diabetics by alleviating hyperglycemia; presumably by enhancing glucose uptake by cells via promoting phosphorylation of insulin receptors. Nevertheless, controversies remain regarding the ameliorative effect of Zn supplementation on oxidative stress in diabetics.

The protective role of supplemental zinc administered as zinc sulfate (100 mg/kg daily for 60 days) on the glycoprotein content and antioxidant enzyme activities in the lung tissue of streptozotocin-induced diabetic rats has been reported (Sacan et al. 2016). The increased glycoprotein content, lipid peroxidation, and AGEs, as well as diminished glutathione concentration and activities of antioxidant enzymes, were significantly countered by the administration of zinc. Thus, this study demonstrates that supplemental Zn offers a beneficial effect on the antioxidant status of lung tissue in diabetes.

A recent animal study has assessed the potential of exogenous Zn intervention in modulating the oxidative stress and cardio protective effects in streptozotocin-induced diabetic rats (Barman and Srinivasan 2017). The oxidative stress markers, activities of antioxidant enzymes, and concentrations of antioxidant molecules, lipid profile, and expression of fibrosis and pro-apoptotic factors in the cardiac tissue were particularly assessed. Diabetic rats treated with supplemental Zn (5- and 10-fold normal requirement) for six weeks showed a significant attenuation of diabetes-induced oxidative stress by up-regulating the compromised activities of antioxidant enzymes and increasing the concentrations of antioxidant molecules (Barman and Srinivasan 2017).

## Beneficial effects of zinc supplementation on secondary complications of diabetes mellitus

Besides being characterized by hyperglycemia, glucosuria, hyperlipidemia, and negative nitrogen balance, diabetes is associated with disabling and life-threatening macro- and micro-vascular complications. Uncompromised diabetes responsible for persistent hyperglycemia leads to chronic secondary complications affecting many organs of the body, both microvascular (neuropathy, retinopathy, and nephropathy) and macrovascular (atheroma). As insulin is stored in $\beta$-cells of the pancreas as a hexamer containing two zinc ions, zinc localized in the islet cells of the pancreas is linked to the synthesis, storage, and secretion of insulin. Oxidative stress precipitates the pathogenesis of diabetic complications. Since Zn is a structural component of several antioxidant enzymes such as superoxide dismutase, its deficiency impairs the synthesis of these enzyme proteins leading to increased oxidative stress. Excretion of large amounts of Zn from the body is reported in the diabetic situation with concurrent hypozincemia and a decrease in tissue Zn concentration. However, it is not clear if the loss of Zn from the body is a result of hyperzincuria or its decreased gastrointestinal absorption or both. Zn deficiency is more common in developing countries, where the prevalence of diabetes is also higher. It has been hypothesized that Zn deficient conditions may aggravate insulin resistance in type-2 diabetes mellitus.

Persistent hyperglycemia in diabetes over the long term is associated with damage, dysfunction, and failure of various organs, especially the nerves, eye lens, kidneys, blood vessels, and heart. Hyperglycemia is proved to be a major perpetrator in the development of microvascular abnormalities comprising neuropathy, retinopathy, nephropathy, and CVD. Increased frequency of diabetic nephropathy has been validated in streptozotocin-induced diabetic rats when Zn deficiency was persuaded by augmented renal excretion or by diet-induced deficiency (Minami et al. 1995). In humans, Zn supplementation (30 mg/day for three months) is shown to protect the advancement of diabetic retinopathy with associated elevated superoxide dismutase (Faure et al. 1995); the decrease in retinopathy is attributed to a beneficial decrease of lipid peroxidation of the retinal polyunsaturated fatty acids. T-lymphocyte response to phytohemagglutinin was observed to be lower in diabetic patients, but not in those with the lowest Zn levels. Zn supplementation to diabetic subjects did not affect the glycosylated hemoglobin level. Strong evidence now points out that oxidative stress-induced inflammatory mechanism would further exacerbate the pathogenesis of diabetic nephropathy (Donath and Shoelson 2011). Zn deficiency is thought to contribute to abnormal immune function in type-2 diabetes mellitus. Hence, it is very logical to believe that oral Zn supplementation may offer a benefit in the management of diabetes mellitus. Indeed several studies have indicated that Zn supplementation in diabetes has a potential hypoglycemic outcome.

Since urinary excretion of Zn is markedly increased in diabetes, replacement of the lost Zn with its oral supplementation should be expected to provide some benefit in this situation. It has been suggested that supplemental Zn could

ameliorate insulin insensitivity in type-2 diabetic patients. Translational studies have proven that Zn supplementation improves insulin levels and has a potential hypoglycemic effect. Human trials have also evidenced the beneficial outcomes of Zn supplementation in both type-1 and type-2 diabetes. However, results of isolated randomized controlled trials are also contradicted in a few studies, suggesting that something more than hyperzincuria is responsible for the abnormalities in Zn metabolism in diabetic patients. Zn supplementation significantly affects the expression of the family of Zn transporters and metallothionein in diabetic patients (Foster et al. 2014). A human study has proved that Zn supplementation can ameliorate albumin excretion, a characteristic of the feature of diabetic nephropathy preceding the development of renal failure.

Several studies have demonstrated that dietary Zn supplementation is effective in the management of diabetes. The antioxidant potential of Zn supplementation is likely to have far-reaching implications in alleviating secondary complications associated with diabetes. Recent animal studies have exhaustively explored the beneficial modulatory potential of Zn supplementation concerning (a) Hyperglycemia and attendant abnormalities in experimental diabetes, (b) Beneficial modulation of compromised antioxidant status, (c) Risk of CVD, diabetic nephropathy, and cataract, and (d) Gastrointestinal health in diabetic condition (Table 1).

## Zinc supplementation alleviates diabetes-induced lipid abnormalities and risk of CVD

Abnormal plasma lipid levels occurring in uncontrolled diabetic patients are key factors in the emergence of microvascular complications. Zn may offer a positive preventive role in atherogenesis. Several clinical studies have evaluated the benefit of Zn supplementation on serum lipids. A meta-analysis (from 24 studies) (Ranasinghe et al. 2015) of all the reported effects of Zn supplementation on serum lipids suggests that Zn supplementation favorably affected plasma lipid profile, which was characterized by reduced cholesterol and triglycerides. In these studies, the dose of Zn supplemented ranged from 15–240 mg/d for a duration of 1 month to 7.5 years. Thus, the meta-analysis of these clinical trials suggests the potential of Zn intervention in reducing the morbidity and mortality associated with atherogenesis.

Diabetic cardiomyopathy is a common secondary complication in type 2 diabetic patients. The relation between Zn status in terms of Zn intake, serum Zn level, and the risk of prospective cardiovascular diseases and type 2 diabetes has been reviewed based on 14 reports (Chu, Foster, and Samman 2016). Higher serum Zn concentration was generally associated with a lower risk of CVD; higher risks of CVD were reported in the vulnerable type 2 diabetic patients. Although the limited available evidence shows no clarity in the association between zinc status and type 2 diabetes-induced CVD risk, more additional studies are warranted in the understanding of the beneficial role of dietary Zn in the prevention of CVD and type 2 diabetes mellitus.

Zinc sulfate supplementation (100 mg/day for 12 weeks) to diabetic patients significantly decreased total cholesterol and triglyceride concentrations in the blood while increasing the HDL-cholesterol (Partida-Hernández et al. 2006). This suggests that Zn treatment could decrease the risk of cardiovascular complications in type-2 diabetic patients. The effect of zinc sulfate supplementation (660 mg per day for twelve weeks) has been evaluated on the blood glucose and lipid profile in type-2 diabetic patients (Afkhami–Ardekani et al. 2008). Zinc sulfate administration produced a significant decrease in HbA1c, triglyceride, and LDL-cholesterol suggesting that zinc sulfate consumption could be beneficial on lipid profile in type-2 diabetic patients. Lipid abnormalities, *viz*., hypercholesterolemia and hypertriglyceridemia were significantly countered by Zn supplementation (5- and 10-fold dietary Zn intervention for six weeks) in STZ-diabetic rats (Barman and Srinivasan 2017). In addition to the attenuation of oxidative stress, supplemental Zn also exerted significant cardio protective effects by moderating the expression of fibrosis and pro-apoptotic factors. Dietary Zn intervention significantly ameliorated the elevated markers of cardiac pathology in circulation and the abnormalities in myocardial tissue architecture of diabetic animals.

While a Zn deficient diet fed for six months promoted the development of diabetic cardiomyopathy in db/db mice (a Type 2 diabetes mellitus rodent model), the animals maintained on Zn supplemented diet showed a significant suppression in the development and progression of diabetic cardiomyopathy (Wang et al. 2017). Cardiac function was significantly decreased in db/db mice. Cardiac dysfunction and hypertrophy observed in db/db mice were accompanied by increased fibrotic factors (collagen accumulation and TGF-$\beta$ level) and inflammatory responses (higher expression of TNF-$\alpha$, IL-1$\beta$, etc.) in the myocardium. While all these effects in diabetic animals were exacerbated by dietary Zn deficiency, the same were alleviated by dietary Zn adequacy and supplements.

## Zinc supplementation alleviates diabetic peripheral neuropathy

An animal study involving streptozotocin-induced diabetic rats revealed that supplemental Zn (5 mg/kg/day as zinc chloride for eight weeks) exerts a protective effect against diabetes-induced peripheral nerve damage by stimulating metallothionein synthesis and lowering oxidative stress (Liu et al. 2014). The neuroprotective effect was evident with Zn supplementation markedly attenuated the diabetes-induced decrease in motor nerve conduction velocity at weeks. Zn supplementation accentuated the increase in the expression of mRNA of metallothionein and attenuated the increase in the mRNA of PARP-1 enzyme in sciatic tissue.

## Alleviation of the progression of diabetic nephropathy by zinc supplementation

Several studies report the beneficial effect of zinc supplementation on diabetic renal disease. In a randomized

**Table 1.** Documented benefits of zinc supplementation in diabetes.

| Diabetic model | Dose of Zinc | Effect demonstrated | References |
|---|---|---|---|
| db/db mice | 300 ppm Zn as $ZnSO_4$ for 6 weeks | Dietary zinc supplementation attenuated hyperglycemia in db/db mice. | Simon and Taylor 2001 |
| Type-2 diabetic patients | 30 mg $ZnSO_4$/ day for 3 months | Zn supplementation resulted in increased serum zinc level, and improved glycemic control as indicated by decreased HbA1c concentration | Al-Maroof and Al-Sharbatti 2006 |
| Type-2 diabetic patients | 30 mg Zn gluconate /day for 6 months | Decreased plasma antioxidant stress without significantly affecting HbA1c and glucose homeostasis | Anderson et al. 2001 |
| Type-2 diabetic patients | 22 mg $ZnSO_4$/day for 4 months) | Improved glycemic control (Reduced HbA1c and post-prandial glucose); lowered serum cholesterol and total cholesterol/HDL cholesterol ratio | Gunasekara et al. 2011 |
| Type-1 diabetic patients | 10 mg elemental Zn as sirup per day for 12 weeks along with vitamin A | Improved serum apoproteins A-I and B, and apoprotein B/apoprotein A-I ratio; No effect on fasting blood sugar and insulin | Shidfar et al. 2010 |
| Type-2 diabetic patients | 30 mg zinc sulfate per day for 6 months | Zinc supplementation improved FBG and HOMA parameters - Beta cell function, insulin sensitivity and insulin resistance | Islam et al. 2016 |
| Prediabetes subjects | 20 mg zinc sulfate per day for 12 months | FBG, HOMA-IR, and hypercholesterolemia were lower; development into type 2 diabetes was lesser | Ranasinghe et al. 2018 |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Mitigation of diabetes-induced zinc dyshomeostasis by its supplementation | Barman, Pradeep, and Srinivasan 2017 |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Alleviation of hyperglycemia and associated metabolic abnormalities in diabetes by zinc supplementation | Barman and Srinivasan 2016 |
| STZ-diabetic rats | 100 mg zinc sulfate/kg daily for 60 days | Prevention of diminished antioxidant status in the lung tissue by zinc supplementation | Sacan et al. 2016 |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Attenuation of diabetes-induced oxidative stress by zinc supplementation | Barman and Srinivasan 2017 |
| Type-2 diabetic patients | 100 mg $ZnSO_4$/day for 12 weeks | Zn supplementation significantly reduced total cholesterol and triglyceride levels, and increased HDL cholesterol in bloodstream | Partida-Hernández et al. 2006 |
| Type-2 diabetic patients | 660 mg $ZnSO_4$/day for 12 weeks | Zinc sulfate administration produced a significant decrease in triglyceride, and LDL-cholesterol, besides HbA1c | Afkhami–Ardekani et al. 2008 |
| db/db Mice | 90 mg zinc sulfate for 6 months | Cardiac dysfunction and hypertrophy were moderated by zinc supplements | Wang et al. 2017 |
| STZ-diabetic rats | 5 mg zinc chloride /kg/day for 8 weeks | Zn supplementation was protective against diabetes-induced peripheral nerve damage by stimulating metallo-thionein synthesis and downregulating oxidative stress | Liu et al. 2014 |
| Type-2 Diabetic patients | 30 mg Zn as 132 mg $ZnSO_4$/d for 3 months | Zinc supplementation ameliorated albumin excretion, a common characteristic of diabetic nephropathy | Parham et al. 2008 |
| Type-2 Diabetic patients | 50 mg Zn as $ZnSO_4$/d for 12 weeks | Zinc supplementation improved glycemic control and also beneficially decreased urinary albumin excretion and inflammation in diabetic nephropathy patients. | Khan et al. 2013 |
| STZ-diabetic rats | 5 mg $ZnSO_4$/kg for 3 months | Protected from diabetes-induced kidney damage via induction of renal MT synthesis | Tang et al. 2010 |
| STZ-diabetic mice | 5 mg $ZnSO_4$/kg for 3 months | Supplemental zinc showed anti-fibrosis effects under hyperglycemic conditions and | Zhang et al. 2016 |

(*continued*)

**Table 1.** Continued.

| Diabetic model | Dose of Zinc | Effect demonstrated | References |
|---|---|---|---|
| | | attenuated tubulointerstitial fibrosis in in the kidneys of diabetic animals | |
| STZ-Diabetic rats | 5 mg $ZnSO_4$/kg/day for 10 weeks | Dietary Zn modulated histomorphological alterations in kidney, particularly renal cortical changes caused by diabetes | Elsaed and Mohamed 2017 |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Alleviation of the progression of diabetic nephropathy by zinc supplementation through a protective influence on oxidative stress induced inflammatory proliferation | Barman, Pradeep, and Srinivasan 2018 |
| STZ-diabetic rats | 30 mg $ZnSO_4$/kg/day for 6 weeks | Supplemental zinc showed protective effect against diabetic kidney damage through stimulation of metallothionein synthesis and regulation of oxidative stress | Özcelik et al. 2012 |
| Type 1 diabetics with early retina lesions | 30 mg zinc sulfate/day for three months | Protected advancement of diabetic retino-pathy | Faure et al. 1995 |
| STZ-diabetic mice | 150 mg zinc sulfate/kg diet | Supplemental zinc showed protective effect on diabetes-induced renal lesion | Yang et al. 2017 |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Amelioration of diabetic cataract by zinc supplementation through modulation of crystallin proteins and polyol pathway | Barman and Srinivasan 2019a |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Ameliorative effect of zinc supplementation on compromised small intestinal health in diabetes | Barman and Srinivasan 2019b |
| STZ-diabetic rats | 5-times and 10-times of normal dietary level for 6 weeks | Improved efficiency in the intestinal absorption of micronutrients in diabetes | Barman and Srinivasan 2018 |
| Diabetic patients with foot ulcer | 220 mg zinc sulfate for 12 weeks | Supplemental zinc improved wound healing by effectively causing decrease in ulcer size | Momen-Heravi et al. 2017 |

double-blind clinical trial, zinc supplementation (30 mg elemental zinc for 3 months) reduced microalbuminuria in type-2 diabetic patients. The authors have attributed the reduced albumin excretion in microalbuminuric type 2 diabetic patients by Zn supplementation to the antioxidant effect of Zn (Parham et al. 2008). In another clinical study, Type-2 diabetic patients with microalbuminuria who were on medication with oral hypoglycemic agents and angiotensin converting enzyme inhibitors were provided as daily supplementation 50 mg Zn as zinc sulfate for 12 weeks (Khan et al. 2013). These authors observed significant decreases in fasting blood glucose, postprandial blood glucose, glycosylated hemoglobin, and hypertriglyceridemia as a consequence of Zn supplementation. Zn supplementation also significantly decreased serum levels of hs-CRP and urinary microalbumin level, suggesting that supplementation of Zn improves not only the effectiveness of oral hypoglycemic agents in glycemic control, but also beneficially decreases urinary albumin excretion and inflammation in diabetic nephropathy patients.

An animal study evaluated if induction of renal metallothionein synthesis by supplemental Zn is protective to diabetes-induced kidney damage (5 mg/kg for three months) (Tang et al. 2010). Streptozotocin-induced diabetic rats were treated with Zn supplementation at 5 mg/kg through drinking water for 3 months. Zn supplementation partially but significantly, prevented the kidney from diabetes-induced renal damage as indicated by significantly attenuated protein excretion, oxidative damage, inflammation and up-regulated expression of the profibrotic mediator – connective tissue growth factor (CTGF) along with significant increases in Zn concentration concomitant with metallothionein expression in renal tubular cells. Thus, this study indicated that Zn supplementation attenuates diabetes-induced renal pathological changes, through the prevention of hyperglycemia-induced CTGF expression by induced metallothionein expression in renal tubular cells.

In another animal study that investigated if dietary Zn modified the diabetes-induced histomorphological alterations in kidneys, supplemental zinc modulated renal cortical changes caused by diabetes in rats (Elsaed and Mohamed 2017). This study was made with groups of STZ-induced diabetic rats that were fed with a standard diet, Zn deficient diet, and Zn supplemented diet. The renal morphological changes (Bowman's capsule, basement membrane, convoluted tubules) in diabetic rats were aggravated by Zn deficiency, while the histopathological alterations were minimal in the cortices of Zn supplemented group. Supplemental Zn is reported to show anti-fibrosis effects under hyperglycemic conditions and attenuate tubulointerstitial fibrosis in diabetic

nephropathy in the kidneys of streptozotocin-treated mice via downregulation of hypoxia-inducible factor-alpha (HIFα) through PI-3K signaling (Zhang et al. 2016).

Zinc preferentially binds to and is a potent inducer of metallothionein under physiological conditions. Zn supplementation (30 mg/kg/day as zinc sulfate for six weeks) has been shown to attenuate metallothionein and oxidative stress in the renal tissue of streptozotocin-induced diabetic rats, and hence protects against the diabetic nephropathy (Özcelik et al. 2012). Zinc supplementation countered the diabetes-induced degenerative morphological changes in the kidney. Immunoreactivity to metallothionein was higher in the tubules of the Zn-supplemented diabetic rats. The concentrations of Zn and metallothionein in the kidney tissue were higher in the Zn supplemented diabetic animals. Thus, exogenous Zn has a protective effect against diabetic nephropathy through stimulation of metallothionein and moderation of the oxidative stress prevalent in diabetes.

A study on streptozotocin-induced diabetic mice that investigated the effects of supplemental Zn (at varying concentrations) on diabetes-induced renal damage suggested that adequate Zn levels are necessary for delaying the progression of diabetic nephropathy (Yang et al. 2017). While the sub-optimal dietary dose of Zn (0.85 mg/kg diet) for 3 months caused Zn deficiency that aggravated the renal damage, dietary Zn at normal (30 mg/kg diet) and higher level (150 mg/kg diet) showed a protective effect on diabetes-induced renal lesion. The renoprotective effects of supplemental Zn were probably mediated through upregulation of Nrf2 and its downstream factors.

Another animal study has explored if supplemental Zn can protect against diabetic nephropathy through modulation of kidney oxidative stress in streptozotocin-induced diabetic rats (Barman, Pradeep, and Srinivasan 2018). Diabetic rats exposed to dietary interventions of Zn supplements for six weeks (5-times and 10-times of normal requirement) exhibited a significant reversal of nephromegaly and creatinine clearance. Stress-induced expression of markers related to the inflammatory process *viz*., inflammatory markers, cytokines, fibrosis factors, and apoptotic regulatory proteins observed in the kidney of diabetic animals were beneficially modulated by zinc intervention. This ameliorative effect was concomitant with elevated anti-apoptosis. A significant reduction was seen in markers of oxidative stress, advanced glycation end products, and expression of the receptor of the glycated products in the kidney tissue. Zn supplementation resulted in countering the over-expression of polyol pathway enzymes in the kidney. Upregulated expression of the glucose transporters, as an adaptation to the increased need for glucose transport in diabetic condition was ameliorated by Zn treatment. The abnormalities in the renal architecture of diabetic animals were predominantly corrected by Zn intervention. Thus, Zn supplementation significantly alleviates diabetic nephropathy, which was exerted through a protective influence on oxidative stress-induced inflammatory proliferation and resultant nephropathy. Zn supplementation particularly alleviated hyperglycemia mediated kidney damage and this involved inhibition of glucose translocation, oxidant stress, and an upsurge in the polyol pathway in the renal tissue (Barman, Pradeep, and Srinivasan 2018).

### *Amelioration of diabetic cataract by zinc supplementation through modulation of crystallin proteins and polyol pathway*

Persistent hyperglycemia leading to non-enzymatic glycation of lens proteins and stimulated polyol pathway in the eye lens has been implicated in the etiology of diabetic cataract. A recent study explored if Zn supplementation protects against diabetes promoted cataractogenesis through moderation of non-enzymatic glycation of lens proteins, elevated polyol pathway, excessive oxidative stress, and heat shock proteins in the eye lens of streptozotocin-induced diabetic rats (Barman and Srinivasan 2019a). Supplemental Zn (5- and 10-times of normal level for six weeks) alleviated the progression and maturation of diabetes-induced cataract. Supplemental Zn was also effective in preventing the reduction in the content of total soluble proteins in the lens. This was accompanied with the alleviation of protein cross-linking through glycation and concomitant expression of the receptor for advanced glycation end products and oxidative stress indicators in the eye lens. Dietary Zn intervention also increased the concentration of heat shock protein particularly of α-crystallin in the eye lens of diabetic rats. Zn supplementation also countered the up-regulation of polyol pathway enzymes and metabolites in the lens of diabetic animals. This animal study thus endorsed the advantage of Zn supplementation in delaying the cataractogenesis in diabetic rats.

### *Ameliorative effect of zinc supplementation on compromised small intestinal health in diabetes*

A recent study explored whether Zn supplementation counters compromised intestinal integrity associated with diabetes through moderation of oxidative stress and suppression of stress-stimulated inflammatory progression in streptozotocin-induced diabetic rats (Barman and Srinivasan 2019 b). Supplemental Zn nurtured diabetic groups (5/10-times of normal level) evidenced a significant reversal in the disruption of intestinal ultra-structure. Brush border membrane (BBM) of diabetic animals which had decreased fluidity associated with increased cholesterol: phospholipid ratio and altered polyunsaturated to saturated fatty acid ratio was beneficially countered in Zn supplementation. The stimulated activity of BBM-bound enzymes suggesting a modulation in membrane dynamics in diabetic condition was moderated in Zn treatment. Higher expression of the lipid oxidative markers, oxidative stress markers, concomitant inflammatory markers, cytokines, fibrosis factors and apoptotic regulatory proteins in the small intestine of diabetic animals were curbed by Zn supplementation. The pathological aberrations in the intestinal architecture of diabetic animals were concomitantly reverted. This study has thus documented that supplemental Zn has a favorable consequence in restricting the compromised intestinal health in diabetes which was exerted through

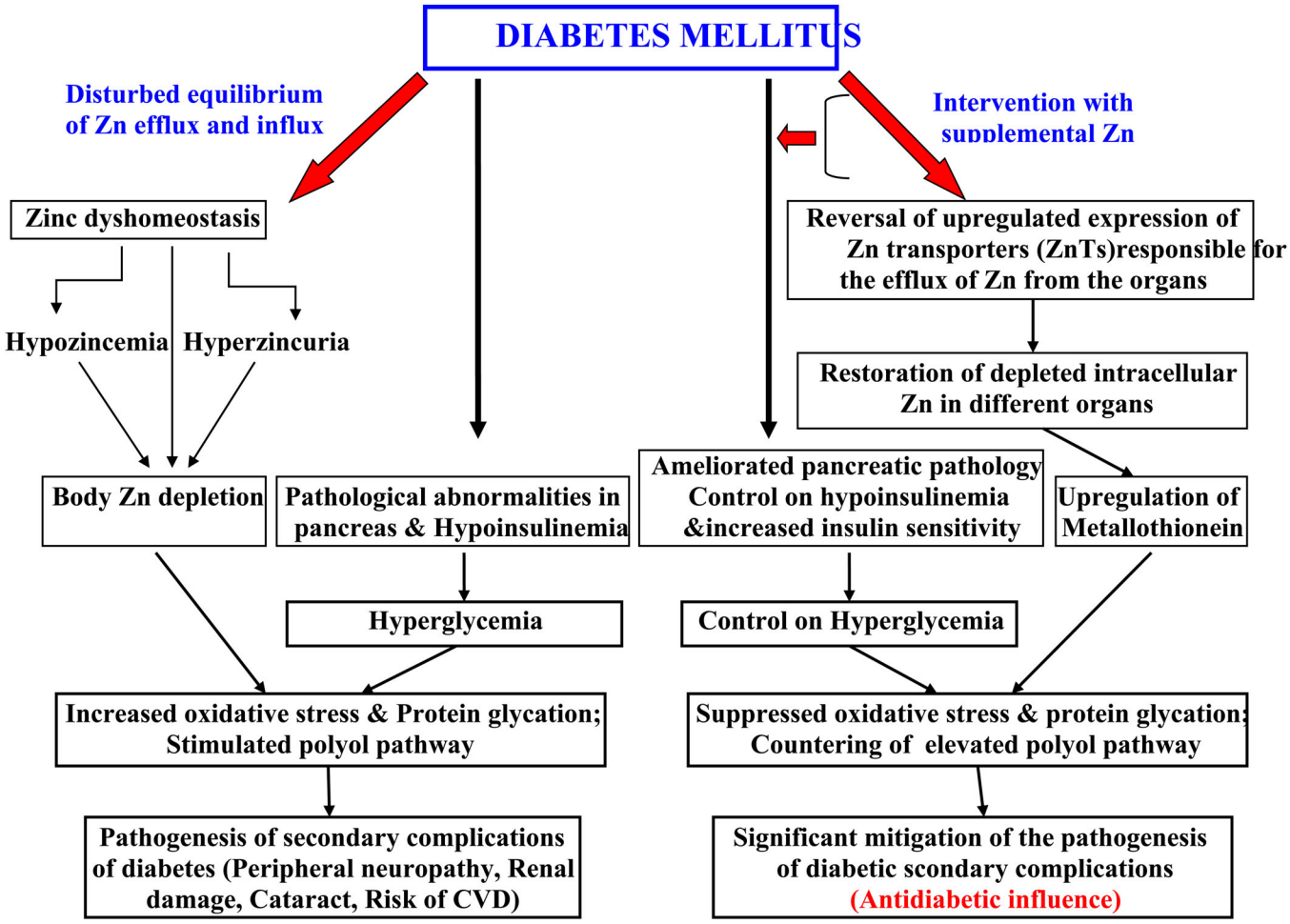


**Figure 2.** Mechanism of antidiabetic influence of supplemental zinc.

a defensive stimulus on oxidative stress-induced cytokines, inflammatory propagation, and subsequent intestine injury.

### *Improved efficiency in the intestinal absorption of micronutrients in diabetes through zinc supplementation*

In continuation of the virtue of Zn supplementation on the restoration of the compromised structural integrity of small intestines in the diabetic situation, a recent study has also documented a significant improvement in the intestinal uptake of micronutrient minerals in streptozotocin-induced diabetic rats maintained on Zn supplementation (Barman and Srinivasan 2018). Since deficiency of Zn is concomitant with other minerals such as iron in diabetic conditions, it is desirable to increase the absorptive efficiency of the small intestinal villi by improving the intestinal health. Intestines isolated from streptozotocin-induced hyperglycemic rats treated for six weeks with supplemental Zn (5-times and 10-times of normal requirement) were examined for possible influence on ex vivo uptake of trace minerals zinc, iron, and calcium. Everted intestinal segments of the duodenum, jejunum, and ileum portions excised from these rats were evaluated for the uptake of iron, zinc, and calcium from the incubation medium containing digesta of a food source as a provider of these trace minerals. While the extent of uptake of these trace minerals predominantly iron was severely compromised in the intestinal segments isolated from diabetic rats as a consequence of loss of functional integrity associated with compromised structural integrity, treatment with supplemental Zn improved the mineral uptake by the isolated intestinal segments, particularly in the jejunal segment and this was attributed to improved expression of applicable membrane-bound transporter protein(s) reported previously. This information on dietary Zn supplementation being evinced to stimulate intestinal absorption of zinc, iron, and calcium, would encourage a dietary approach to counter progressive dyshomeostasis of these trace minerals prevalent in diabetes.

Zinc is considered to be relatively nontoxic, particularly when consumed orally. However, overt toxicity symptoms may manifest with extremely high zinc intakes (Fosmire 1990). Excess zinc ingestion is one of the causes of copper deficiency (Willis et al. 2005). Even such intakes of Zn at low amounts but well above the Recommended Dietary Allowance (say, 100–300 mg Zn/day) can induce copper deficiency with attendant symptoms of anemia and impaired immune function and also adversely affect the ratio of LDL to HDL (Fosmire 1990). It should be noted that the 5- and 10-times RDA dose of supplemental Zn did not develop any oxidative stress in experimental diabetes, and certainly was not damaging to any of the organs and also did not

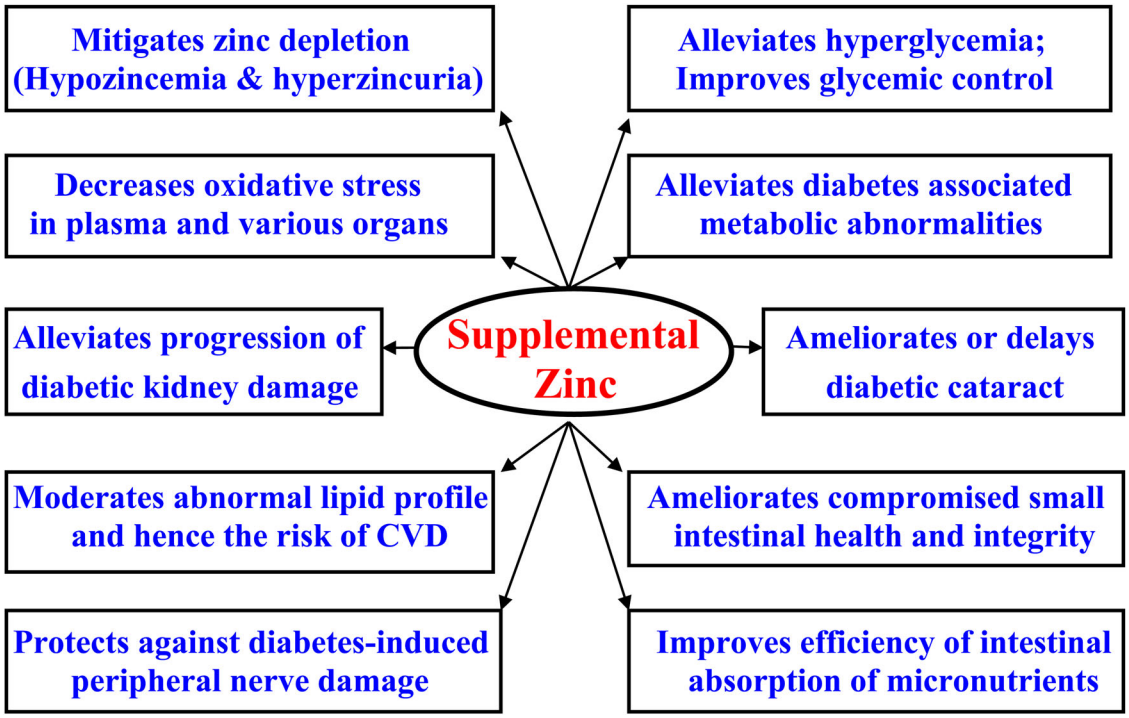


**Figure 3.** Proven benefits of supplemental zinc in diabetes.

adversely affect LDL to HDL (Barman and Srinivasan 2016; Barman and Srinivasan 2017). These authors have chosen this 5 and 10 x RDA dose of Zn supplementation only to offset the heavy loss of Zn from the body during diabetes. Although the impact of Zn supplementation on copper status has not been studied in the above animal studies, a possibility that it could lead to harmful copper deficiency in humans as a result of a long term Zn supplementation cannot be ruled out.

### *Beneficial effect of zinc supplementation on wound healing in diabetes*

In a placebo-controlled study performed to examine the beneficial effect of Zn supplementation on wound healing in patients with diabetic foot ulcer, Zn supplementation (50 mg elemental Zn as 220 mg zinc sulfate) for 12 weeks is understood to have beneficial effects on ulcer size (Momen-Heravi et al. 2017).

## Efficacy of zinc compounds for supplementation

While simple inorganic compounds of Zn, such as $ZnSO_4$ and $ZnCl_2$ are conventionally examined in these studies for offsetting the loss of this micro mineral in the diabetic situation, novel zinc coordination compounds (Sakurai, Yoshikawa, and Yasui 2008) as well as zinc gluconate could be more efficient in the clinical situation as they may necessitate lower dosages for the same effect and thus any problem of Zn toxicity would be overcome. Zinc oxide nanoparticles, a novel agent to achieve Zn delivery have great implications in therapeutic applications in diabetes mellitus patients (Tang 2019). The use of zinc oxide nanoparticles to successfully restore the structure and function of pancreatic islet cells in high fat-fed and streptozotocin-induced diabetic Wistar rats has been reported (El-Gharbawy, Emara, and Abu-Risha 2016). The use of zinc oxide nanoparticles along with Vildsagliptin (a standard antidiabetic drug) was found to have a synergistic therapeutic effect on diabetes. Cardio-protective effects of zinc oxide nanoparticles evaluated in streptozotocin-induced diabetic rats showed higher efficacy as compared to zinc sulfate (30 mg/kg given for 8 weeks) (Asri-Rezaei et al. 2017). The recovery of cardiac damages by zinc nanoparticles was evidenced by serum lipid profile, atherogenic index, cardiac oxidant status, apoptosis indices, and histopathological features. These studies suggest that Zn nanoparticles have the potential for a better Zn delivery to reap the benefit of this supplemental micronutrient in diabetic patients.

## Conclusion

Diabetes authenticates Zn dyshomeostasis in the body; contrariwise, Zn deficiency increases the risk of diabetes and its complications. A recent animal study has documented that dietary Zn supplementation (5-times/10-times of RDA) significantly controls diabetes-induced Zn dyshomeostasis. This is achieved through regulation of the tissue-specific Zn transporters and protective influence on the oxidative stress through metallothionein proliferation. Animal studies have also shown that Zn supplementation significantly ameliorates hypoinsulinemia, hyperglycemia and its associated metabolic abnormalities, and oxidative stress in tissues. Dietary Zn supplementation significantly helps in the control of diabetic nephropathy, which is exerted through a protective influence on oxidative stress-induced cytokines,

inflammatory proliferation, and consequent renal injury. The results also endorsed the benefit of Zn supplementation in exerting antiglycating influence and down-regulating polyol pathway enzymes thus contributing to defer cataractogenesis in diabetic rats (Figure 2). Supplemental zinc intervention is also understood to exert a favorable effect on the restoration of structural integrity of small intestines in diabetic situation, which is mediated through a defensive stimulus on oxidative stress-induced cytokines, inflammatory propagation, and subsequent tissue injury. Supplemental zinc is also reported to exert a promoting stimulus on the intestinal absorption of zinc, iron and calcium, which could encourage a dietary approach to counter the dyshomeostatic state of these trace elements prevalent in diabetes.

Thus, Zn supplementation may offer a promising potential for clinical application in the management of diabetic hyperglycemia and its long term consequences. The encouraging basic information generated by recent investigations on the positive impact of Zn supplementation suggests an effective dietary strategy that may prove advantageous in the management of all the secondary complications of diabetes (Figure 3). Because of the serious challenge of metabolic disorders caused by oxidative stress in diabetes, further more clinical studies are necessary to fully understand the beneficial role played by Zn supplementation against oxidative stress in type 2 diabetes mellitus prognosis. Further knowledge on this would drive forward the strategic development of zinc nutrition for the preventive treatment of diabetes-related disorders.

## Conflict of interests

The authors declare that there are no conflicts of interest.

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