The Role of Zinc Homeostasis in the Prevention of Diabetes Mellitus and Cardiovascular Diseases

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Zinc is an essential micronutrient for human health and is involved in various biological functions, such as growth, metabolism, and immune function. In recent years, research on intracellular zinc dynamics has progressed, and it has become clear that zinc transporters strictly control intracellular zinc localization, zinc regulates the functions of various proteins and signal transduction pathways as a second messenger similar to calcium ions, and intracellular zinc dyshomeostasis is associated with impaired insulin synthesis, secretion, sensitivity, lipid metabolism, and vascular function. Numerous animal and human studies have shown that zinc deficiency may be associated with the risk factors for diabetes and cardiovascular diseases (CVDs) and zinc administration might be beneficial for the prevention and treatment of these diseases. Therefore, an understanding of zinc biology may help the establishment of novel strategies for the prevention and treatment of diabetes and CVDs. This review will summarize the current knowledge on the role of zinc homeostasis in the pathogenesis of diabetes and atherosclerosis and will discuss the potential of zinc in the prevention of these diseases.

Key words: Zinc, Zinc transporter, Insulin resistance, Diabetes, Endothelial function, Atherosclerosis, Cardiovascular diseases

Introduction

Diabetes and cardiovascular diseases (CVDs) are the leading cause of death worldwide, and diabetes is well known to be deeply associated with the pathogenesis of CVDs. The long-term persistence of diabetic conditions, such as hyperglycemia, impaired insulin action, increased advanced glycation end products (AGEs), and inflammation, is involved in vascular dysfunction and atherosclerosis. Since nutritional status plays a significant role in modulating these risk factors for CVDs, understanding the pathophysiology of metabolic disorders from the perspective of nutritional biology is critical in establishing new preventive strategies for CVDs.

Zinc is a micronutrient that plays a vital role in the regulation of whole-body metabolism, growth, and the immune system. Zinc is broadly distributed in all body tissues, with ~85% of the whole-body zinc in muscle and bone tissues. Another 11% is distributed in the skin and liver, and the remaining 2%–3% is in all the other tissues, including blood and blood vessels. Zinc deficiency is well known to be involved in various disorders in the whole-body, such as growth failure, immune disorders, and dysgeusia. Furthermore, zinc deficiency also increases the risk for diabetes and CVDs. Recent findings have shown that intracellular zinc homeostasis regulated by zinc transporters is involved in the regulation of insulin synthesis, secretion, sensitivity, and vascular function. This review will discuss the role of impaired intracellular zinc homeostasis in the pathogenesis of diabetes and atherosclerosis and whether zinc supplementation is beneficial for the prevention of these diseases.

1. Intracellular Zinc Homeostasis

Zinc is a divalent cation and can bind to ~10% of all proteins found in the human body and functions...
as a cofactor of more than 300 enzymes. Since zinc is a non-redox metal ion, zinc can bind various proteins stably and can provide the catalytic activity of enzymes and structural stability of proteins^{9}. Typically, intracellular zinc is distributed in the nucleus (30%–40%) and cytoplasm, organelles and vesicles (~50%). Furthermore, intracellular zinc is distributed in four main pools related to the regulation of zinc homeostasis: 1) zinc bound to metalloenzymes as structural components or as a cofactor, 2) bound to metallothionein (MT), 3) stored in intracellular organelles (e.g., nucleus, Golgi apparatus, and endoplasmic reticulum (ER)) and vesicles, and 4) cytoplasmic free zinc^{10}. Under normal conditions, cytoplasmic free zinc levels are maintained at a quite low concentration (pM–low nM levels). This is achieved by MT in the cytoplasm and zinc transporters in the plasma membrane and organelle membranes. In the cytoplasm, MT binds with zinc to reserve, chelate, and buffer zinc. Furthermore, zinc transporters tightly regulate the movement of zinc into or out of cells, intracellular organelles, and vesicles. Free zinc binds various proteins and modulates their functions, thus intracellular zinc compartments are thought to be regulated strictly.

2. Zinc Transporters and Zinc Signaling

The mobilization of zinc into or out of cytosol/intracellular organelles are controlled by two zinc transporter families, the Zn transporter (ZnT)/SLC30A family and the Zrt/Irt-like protein/solute carrier family 39 (ZIP/SLC39A)^{11}. ZnTs are zinc exporters that transport zinc into extracellular matrix or organelles from the cytoplasm, whereas ZIPs are zinc importers that transport zinc into the cytoplasm from extracellular matrix and organelles. There are nine ZnT and 14 ZIP transporters encoded in the human genome, and these zinc transporters are located in the plasma membrane and organelle membranes and maintain intracellular zinc distribution and zinc homeostasis (Fig. 1). The mutation in several zinc transporters has already been demonstrated to be associated with genetic diseases in humans. For example, the loss-of-function mutation of ZIP4 and ZIP13 is responsible for acrodermatitis enteropathica and spondylocheirodysplastic Ehlers-Danlos syndrome, respectively^{12, 13}. Moreover, there is growing evidence that zinc ion mobilized by the zinc transporter acts as a signaling molecule similar to a calcium ion and regulates various cell signaling pathways and many biological functions^{11}. Therefore, knowledge about zinc biology is significant for understanding cell biology and pathophysiology.

3. Role of Zinc Homeostasis in Glucose Metabolism

3-1. Role of Zinc in Insulin Secretion

Pancreatic β cells have a high zinc content^{14}, and insulin secretory granules have the highest concentration of zinc within β cells^{15}. It has been
shown that zinc plays a significant role in the crystallization of insulin; thus, the association between zinc homeostasis in β cells and impaired insulin secretion have attracted attention\(^6\). Insulin exists in pancreatic β cells as a hexamer consisting of two zinc ions and six insulin molecules, and this hexamer exists as a crystal under a special environment in insulin granules\(^16, 17\). Proinsulin synthesized in β cells is modified in the ER and then forms a dimer in the Golgi apparatus. The two proinsulin dimers interact with their respective HisB10 residues via two zinc ions to form a proinsulin tetramer. It then binds to the dimer unit to form a hexamer consisting of two zinc ions and six proinsulin molecules. When the zinc–proinsulin hexamer moves into the insulin granule, it is converted into a zinc–insulin hexamer, and the zinc–insulin hexamer forms a crystal structure under acidic conditions in the insulin granules. Both human and animal studies showed that zinc contents in the pancreas were lower in diabetic patients and animals than in healthy controls\(^18\). In addition, zinc deficiency decreased insulin secretory granules in β cells\(^19\) and impaired glucose-induced insulin secretion in rats\(^20\). These findings suggest that zinc is required for normal insulin secretion and that zinc deficiency in pancreatic β cells is involved in abnormal insulin secretion.

### 3-2. Role of Zinc Transporters in Insulin Secretion

The genome-wide association study revealed that single-nucleotide polymorphism in the SLC30A8 gene encoding ZnT8, resulting in the replacement of tryptophan-325 with arginine (Arg325Trp), are associated with an increase in the risk of type 2 diabetes\(^21\). In fact, meta-analysis has shown that ZnT8 Arg325Trp polymorphism increased the risk of type 2 diabetes by 14%\(^22\). In addition, another human study found that this polymorphism decreases glucose-stimulated insulin secretion\(^23\). ZnT8 is a zinc transporter highly expressed in the plasma membrane of insulin granules of pancreatic β cells and is considered to transport zinc from the cytosol into insulin granules in β cells\(^24\). Analysis with ZnT8 KO mice revealed that zinc transport into insulin granules via ZnT8 plays a critical role for the crystallization of insulin\(^25, 26\). On the other hand, Tamaki et al. have reported that glucose-stimulated insulin secretion was slightly increased in ZnT8 KO mice than in control mice, despite the decreased circulating insulin levels\(^27\). This discrepancy appears to be explained by increasing insulin clearance in the liver of ZnT8 KO mice\(^28\). Similar to ZnT8 KO mice, ZnT8 Arg325Trp polymorphism in humans has also been shown to enhance insulin clearance\(^29\). These findings suggest that mutations in the ZnT8 gene might cause impaired glucose tolerance through abnormal insulin clearance rather than through abnormal insulin secretion, resulting in an increased risk of diabetes. However, \textit{in vitro} study using Min6 cells (mouse pancreatic β cell line) found that the overexpression of \textit{hZnT8} polymorphisms enhances the activity of ZnT8, resulting in the enhancement of zinc transport into β cells\(^27\). In addition, recent reports have shown that ZnT8 gene polymorphism is associated with high zinc levels in human islets\(^28\). However, zinc levels and proinsulin levels in islets were reduced in \textit{hZnT8} transgenic mice that overexpress the Arg325Trp polymorphism\(^29\). Thus, the influence of Arg325Trp polymorphism on ZnT8 activity seems to be controversial.

Regarding other zinc transporters, ZnT3 and ZnT7 are associated with β cell function. Smidt et al. have shown the presence of ZnT3 in insulin granules of INS-1E cells (rat pancreatic β cell line), and have demonstrated that knockdown of ZnT3 in INS-1E cells decreases the expression and secretion of insulin\(^30\). Furthermore, streptozotocin-treated ZnT3 KO mice were more susceptible to glucose intolerance\(^30\), suggesting that ZnT3 expressed in insulin granules is associated with insulin synthesis and secretion. However, overexpression of ZnT3 in INS-1E cells resulted in decreased insulin synthesis and secretion\(^31\). The authors explained this discrepancy as shown by the alteration of ZnT8 expression. They found the inverse correlation between ZnT3 and ZnT8 expression in INS-1E cells, suggesting that the upregulation of ZnT3 decreases insulin contents and secretion due to the decreased ZnT8 expression in β cells\(^31\). Regarding ZnT7 located in the Golgi apparatus, Huang et al. reported that the overexpression of ZnT7 in RINm5F cells (rat insulinoma cells) increased insulin contents and insulin secretion, suggesting a positive regulation of ZnT7 on insulin synthesis and secretion\(^32\).

In addition to these zinc transporters, the relationship between Zip5 (located in the plasma membrane of β cells) and insulin secretion has been reported recently. β-cell-specific Zip5 KO mice exhibited a marked decrease in zinc contents in β-cells and impaired insulin secretion through decreased Sirt1 and PGC-1α and downregulation of GLUT2\(^33\). This finding indicates that zinc transport into β cells via Zip5 controls insulin secretion through the increase in glucose uptake via GLUT2.

![Fig.2](image-url)

\textbf{Fig.2} shows the proposed role of zinc transporters in insulin secretion based on the above findings. Although the detailed roles of some of these zinc transporters are still unclear, it is certain that the regulation of zinc distribution by the zinc transporters...
plays an important role in insulin secretion.

3-3. Role of Zinc in Insulin Signaling

Insulin controls blood glucose levels by promoting glucose uptake into insulin-sensitive organs, such as the skeletal muscle, liver, and adipose tissues, and promoting glycogen synthesis and inhibiting gluconeogenesis in the liver. Zinc is known to regulate insulin signaling through its insulin-mimetic actions\(^{34}\). Zinc can enhance the phosphorylation of insulin receptors and can activate phosphatidylinositol 3 kinase (PI3K) and protein kinase B (Akt)\(^{34}\). As one of these molecular mechanisms, zinc has been demonstrated to inhibit the activity of protein tyrosine phosphatase (PTPase) 1B, which is an enzyme that inhibits insulin action through dephosphorylation of the β subunit of the insulin receptor\(^{35}\). Zinc can also activate Akt through the inhibition of phosphatase and tensin homolog (PTEN), an enzyme that promotes the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate (PIP3)\(^{36}\). Furthermore, zinc has also been reported to activate Akt and PI3K directly\(^{37}\). Wu et al. demonstrated that zinc treatment activates Akt signaling and enhances the translocation of GLUT4 to the plasma membrane, leading to the upregulation of glucose uptake in L6 myotubes\(^{38}\). Collectively, these findings suggest that zinc can modulate insulin signaling through several molecular mechanisms.

Zinc can also regulate glycogen synthesis and gluconeogenesis. Zinc inhibits the activation of glycogen synthase kinase 3 (GSK3), resulting in an increase in glycogen synthesis\(^{39}\). Zinc has also been reported to inhibit the activity of transcription factor forkhead box protein O1 (FoxO1)\(^{40}\). FoxO1 is a transcription factor that regulates various cell functions, including gluconeogenesis\(^{41}\). Zinc promotes phosphorylation of FoxO1 and its translocation to the cytoplasm from the nucleus, resulting in an inactivation of FoxO1\(^{40}\).

Taken together, these findings suggest that zinc can regulate glucose metabolisms by promoting glucose uptake and glycogen synthesis and inhibiting gluconeogenesis (Fig. 3).

3-4. Zinc Transporters and Insulin Resistance

Table 1 shows the summary on the relationship between zinc transporters and insulin resistance. ZnT7 is in the Golgi apparatus and secretory vesicles and transports zinc into the Golgi apparatus and the vesicles. ZnT7 KO mice were more susceptible to a high-fat diet-induced glucose intolerance and insulin resistance due to the downregulation of Akt signaling and impaired fatty acid metabolism in the muscle\(^{42, 43}\). ZiP7, a zinc transporter in the ER and Golgi apparatus, has also shown to be associated with the regulation of glucose metabolism. ZiP7 knockout using specific siRNA in skeletal muscle cells resulted in the inhibition of Akt phosphorylation and the reduction of insulin-stimulated glycogen synthesis\(^{44}\). These findings suggest that ZnT7 and Zip7 positively regulates insulin signaling and glucose metabolism in the skeletal muscles.
KO mice also exhibited the enhancement of glycogen synthesis and inhibition of gluconeogenesis. As the underlying mechanism, it has been suggested that ZiP14-mediated zinc transport to the early endosomes suppresses insulin signals through the degradation of insulin receptors in the early.

On the other hand, zinc transport via Zip14 is suggested to negatively control the insulin signaling in the liver. An animal study using Zip14 KO mice showed that Zip14 deficiency improved hepatic insulin resistance in mice fed with a high-fat diet, despite a marked decrease in zinc contents in the liver. Zip14 KO mice also exhibited the enhancement of glycogen synthesis and inhibition of gluconeogenesis. As the underlying mechanism, it has been suggested that Zip14-mediated zinc transport to the early endosomes suppresses insulin signals through the degradation of insulin receptors in the early.

Table 1. Summary of basic research on the relationship between zinc transporter and insulin resistance

| Zinc transporter (Subcellular Localization) | Experimental design | Phenotypes | Zinc contents in target tissues or cells | Reference |
|---------------------------------------------|---------------------|------------|----------------------------------------|-----------|
| ZnT7 (Golgi apparatus, Vesicles)            | Global KO mice fed with high-fat diet (in vivo) | More susceptible to diet-induced glucose intolerance and insulin resistance in skeletal muscle tissues | Decreased (in skeletal muscle tissues) | Huang L et al. J Biol Chem 2012 (42), J Biol Chem 2018 (43) |
| ZnT7 (Golgi apparatus, Vesicles)            | Global KO mice fed with normal diet (in vivo) | Suppressed body weight gain and fat accumulation through impaired insulin signaling and glucose uptake in subcutaneous adipose tissues | Decreased (in subcutaneous adipose tissues) | Tepamormdech S et al. FEBS J 2016 (46) |
| Zip7 (ER)                                   | Knockdown in skeletal muscle cells (in vitro) | Impaired Akt signaling and glucose uptake | Decreased (in skeletal muscle cells) | Myers SA et al. PLoS One 2013 (44) |
| Zip13 (Golgi apparatus)                     | Global KO mice fed with high-fat diet (in vivo) | Improved diet-induced obesity and insulin resistance, enhanced beige adipocyte differentiation and energy expenditure | NA | Fukunaka A et al. PLoS Genet 2017 (47) |
| Zip14 (Plasma membrane, Early endosome)     | Global KO mice fed with high-fat diet (in vivo) | Improved hepatic insulin resistance | Decreased (in liver) | Aydemir TB et al. J Biol Chem 2016 (45) |
| Zip14 (Plasma membrane, Vesicles)           | Global KO mice fed with normal diet (in vivo) | Enhanced adipose tissue inflammation | Increased (in adipose tissues) | Troche C et al. Am J Physiol Endocrinol Metab 2016 (48) |
3-5. Blood Zinc Levels in Abnormal Glucose Metabolism

Several studies showed lower blood levels of zinc in patients with type 2 diabetes45-51, and a recent meta-analysis also revealed that whole blood zinc levels are decreased in patients with type 2 diabetes than in healthy subjects without differences in zinc intake52. This analysis suggests that the condition of type 2 diabetes is associated with decrease in whole blood zinc levels. Another study also reported that plasma zinc levels were decreased in patients with type 2 diabetes with poor glycemic control, whereas urinary zinc excretion is increased, suggesting that glucose abnormal metabolism affects zinc homeostasis partly due to the increase in the urinary loss of zinc53. Type 1 diabetic condition may also affect zinc homeostasis. Forte et al. examined the difference in the whole blood zinc levels between patients with type 1 diabetes (n = 196) and control subjects (n = 59)54. Whole blood zinc levels in diabetic patients were significantly lower than in those of control subjects54. Furthermore, some reports also have shown that plasma zinc/copper ratio was decreased in patients with both type 1 and type 2 diabetes than in healthy subjects55, 56.

3-6. Is Lower Zinc Level A Risk for Diabetes?

Animal Studies

A zinc-deficient diet augmented hyperglycemia and increased circulating glucagon levels in type 2 diabetic model mice (leptin receptor-deficient db/db mice), suggesting that lower zinc status aggravates glucose metabolism57. A study using a type 1 diabetic model mice also showed that dietary zinc restriction promoted degradation of the endocrine pancreas, resulting in augmented hyperglycemia58. Given these animal studies, zinc deficiency is likely to be associated with the development of both type 1 and type 2 diabetes.

Human Studies

A prospective cohort study that investigated the association between zinc intake and risk of type 2 diabetes in 82,297 women in the United States revealed that women with higher zinc intake showed a lower risk of type 2 diabetes59. Furthermore, a negative correlation between serum zinc levels and the risk for type 2 diabetes was found in a case control study that included 1,796 participants (218 newly diagnosed patients with impaired glucose regulation, 785 newly diagnosed patients with type 2 diabetes, and 793 healthy subjects)60. In an analysis of patients with type 1 diabetes, lower zinc levels were associated with the increased incidence of type 1 diabetes61. Interestingly, lower zinc levels of drinking water have been shown to be associated with an increased risk of type 1 diabetes in children62, 63. These findings suggest that lower zinc levels is a risk factor for the development of both type 1 and type 2 diabetes. In contrast, some studies have shown that zinc supplementation for 14 weeks attenuated glucose and lipid metabolic abnormalities in mice fed with a high-fat diet65. In addition, dietary zinc supplementation for 6 weeks attenuated hyperglycemia and hyperinsulinemia in type 2 diabetic db/db mice66.
Human Studies

Regarding humans, a meta-analysis revealed that zinc supplementation improves glycemic parameters (fasting blood glucose levels and HbA1c) as well as the serum lipid profile in patients with type 2 diabetes\(^{67, 68}\). Another meta-analysis including 14 randomized control trials (\(n = 3,978\) subjects) has shown that zinc supplementation markedly reduced the glucose levels in subjects with chronic metabolic diseases (types 1 and 2 diabetes mellitus, metabolic syndrome, and obesity) compared with healthy subjects\(^{69}\). Furthermore, recent meta-analysis also showed the beneficial effect of zinc on glycemic parameters in patients with type 2 diabetes\(^{70, 71}\). Taken together, these findings strongly suggest that zinc supplementation is beneficial for the prevention or treatment of type 2 diabetes. In contrast, there is little evidence of the effects of zinc on type 1 diabetes. In 2005, de Sena et al. examined the effect of zinc supplementation in 20 children with type 1 diabetes\(^{72}\). Zinc supplementation had no effects on blood glucose levels in children with type 1 diabetes\(^{72}\). Furthermore, large-dose zinc supplementation induced an undesirable elevation of HbA1c in 14 patients with type 1 diabetes\(^{73}\). Further studies with a large sample size would be necessary to elucidate the effect of zinc supplementation on type 1 diabetes.

4. Role of Zinc Homeostasis in CVDs

4-1. Zinc and Endothelial Function

Nitric oxide (NO) produced from vascular endothelial cells acts on vascular smooth muscles to cause vasodilation, resulting in an antihypertensive effect\(^{74}\). NO is also known to have various physiological activities, such as the inhibitory action of platelet aggregation and vascular smooth muscle cell (VSMC) proliferation\(^{75}\). NO is produced from arginine by the catalytic action of endothelial NO synthase (eNOS). The decrease in NO production caused by the decrease in eNOS expression or activity is deeply involved in the development of arteriosclerosis\(^{75}\). Numerous clinical and animal studies have confirmed that metabolic disorders, including type 1 and type 2 diabetes, cause vascular endothelial dysfunction with reduced eNOS activity and NO production\(^{76-78}\).

It is known that eNOS exerts its function by forming a dimer, and zinc is essential for the formation of the dimer\(^{79, 80}\). Although it is not clear whether increasing or decreasing zinc in vascular endothelial cells directly regulates eNOS activity, treatment with N,N,N,N-Tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN), a zinc chelator, converted eNOS dimer into eNOS monomer in endothelial cells, suggesting that intracellular zinc regulates eNOS function\(^{80}\). Oxidizing agents (e.g., peroxynitrite (ONOO\(^{-}\))) that are increased in pathological condition such as diabetes rapidly release zinc from eNOS\(^{80}\). Furthermore, increased zinc release from eNOS and decreased eNOS dimer have been shown in diabetic model animals\(^{80}\). Therefore, the increased zinc release from eNOS by changes in intracellular redox state may be one of the mechanisms of suppressing NO synthesis in endothelial cells in a diabetic condition. It has also been reported that zinc deficiency during fetal and postnatal periods causes decreased expression and activity of eNOS in rats\(^{81}\). This finding indicates that intracellular zinc deficiency may impair endothelial NO synthesis and function through the reduction of eNOS activity\(^{8}\).

On the other hand, NO may regulate zinc homeostasis in vascular endothelial cells. NO has been reported to release zinc bound to intracellular metallothionein and mobilize free zinc into the cytoplasm of endothelial cells\(^{82, 83}\). Given this, NO may increase the concentration of free zinc in vascular endothelial cells, resulting in a positive feedback regulation of NO production through the enhancement of eNOS activity by zinc.

Zinc also exhibits anti-apoptotic, anti-inflammatory, and anti-oxidative action in vascular endothelial cells\(^{7}\). Meerarani et al. reported that zinc deficiency induced apoptosis in vascular endothelial cells via the activation of caspase 3, whereas zinc supplementation suppressed them\(^{84}\). Connell et al. reported that supplementation with physiological concentrations of zinc suppressed the tumor necrosis factor-\(\alpha\)-induced the inflammatory response in vascular endothelial cells\(^{85}\). Zhuang et al. also reported that zinc supplementation suppressed AGE-induced decrease in NO production and eNOS activity and increase in nuclear factor-\(\kappa\)B (NF-\(\kappa\)B) activity in vascular endothelial cells\(^{86}\).

Collectively, these findings indicate that zinc has protective effects against endothelial dysfunction through the increased eNOS activity and NO production and suppressed apoptosis and inflammation (Fig. 4).

4-2. Zinc and VSMCs

Zinc has been suggested to regulate the proliferation and apoptosis of VSMCs. Chronic zinc deficiency promoted the proliferation of VSMCs through the suppression of c-Jun N-terminal kinase (JNK) signals in rats\(^{87}\). Zinc deficiency for 2 weeks enhanced apoptosis through the activation of extracellular signal-regulated kinase (ERK) in the
vascular smooth muscle layer of blood vessels in rats\(^9\). As the underlying mechanism, it has been suggested that the enhanced dephosphorylation of Bcl2-associated agonist of cell death (BAD) protein is due to the activation of calcineurin by zinc deficiency. Zinc may also suppress the calcification of VSMCs. In cultured VSMCs, zinc treatment suppressed osteo-/chondrogenic trans-differentiation and calcification induced by high phosphate stimulation through the suppression of NF-\(\kappa\)B activity\(^8\). These findings suggest that zinc can prevent atherosclerosis and CV
d through the inhibition of the proliferation, apoptosis, and calcification of VSMCs.

4-3. Zinc Transporters and Vascular Cell Function

Recent study has revealed the expression patterns of zinc transporters in human vascular endothelial cells and pulmonary VSMCs\(^9\). The expression patterns of zinc transporters in vascular endothelial cells and VSMCs were very similar, and the expressions of ZnT7, ZnT9, Zip9, and Zip10 were the highest at the mRNA level. On the other hand, the expressions of ZnT2, ZnT3, ZnT10, Zip2, Zip5, and Zip12 were exceptionally low, and the expression of ZnT8 and ZiP4 was not observed. This study also showed that zinc deficiency in vascular endothelial cells and VSMCs significantly increased Zip2 and Zip12 expression. Immunocytochemical analysis revealed that increased expression of Zip2 in vascular endothelial cells due to zinc deficiency was observed intracellularly but not in the cell membrane, suggesting that Zip2 provided zinc to the cytoplasm of intracellular vesicles in endothelial cells. On the other hand, the increased expression of Zip12 was observed on the plasma membrane in endothelial cells\(^9\). These findings suggest that Zip2 and Zip12 play a significant role in regulating zinc homeostasis in vessels.

Dysregulation of these two transporters may be associated with vascular diseases. Mutations of Zip2 genes in humans are associated with the development of carotid artery diseases in the elderly\(^9\). Regarding Zip12, it has been reported that the increased expression of Zip12 is observed in VSMCs in hypoxia-induced pulmonary arteries in rats\(^9\). Furthermore, deletion of Zip12 suppressed the increase in free zinc content and the proliferation of VSMCs under hypoxic conditions, resulting in the prevention of pulmonary hypertension in rats\(^9\). These findings suggest that zinc mobilization into VSMCs via Zip12 is involved in the pathogenesis of pulmonary hypertension.

4-4. Zinc Sensing Receptor (ZNR)/GPR39 and Vascular Cell Function

In addition to zinc transporters, recent finding suggests that G protein-coupled receptor 39 (GPR39), a ZNR in the cellular membrane, is associated with the regulation of endothelial cell function. GPR39 is regulated by the change in extracellular zinc ion and modulates several signaling pathways\(^9\). Zhu et al. reported that knockdown of GPR39 in vascular endothelial cells abolished zinc-promoted cell survival, proliferation, and angiogenesis through the downregulation of the \(\text{G}_\text{aq}-\text{Phospholipase C}\) pathway\(^9\). This finding suggests that extracellular
zinc regulates endothelial cell function through GPR39, and that changes in extracellular zinc levels directly affect endothelial function.

4-5. Blood Zinc Levels and CVDs

**Animal Studies**

Animal studies using zinc-restricted diets have been conducted to clarify the relationship between zinc levels in the body and the risk factors for CVDs and the development of CVDs. Dietary zinc restriction affected cholesterol homeostasis, such as the reduction of circulating high-density lipoprotein-cholesterol, apoE, and apoA in rats95). An animal study using atherogenic mice has shown that chronic dietary zinc restriction for over 6 months resulted in increased circulating low-density lipoprotein (LDL)-cholesterol levels and circulating markers of vascular inflammation and more aortic plaque formation than in mice fed with zinc-adequate diet96), suggesting that lower zinc levels is associated with vascular inflammation and increased risk for CVDs.

**Human Studies**

Regarding humans, several studies that analyze the association between plasma/serum zinc levels and the risk of CVDs has been reported97-106). A systematic review of a prospective cohort study showed that higher zinc serum levels are associated with a lower risk of CVDs106). Some studies have shown that lower zinc levels were inversely correlated with CVDs98, 100, 101). Furthermore, a meta-analysis of 13 case studies demonstrated that there are significant associations between zinc deficiency and the incidence of myocardial infarction107). These evidence indicate that lower blood zinc levels are associated with an increased risk of CVDs.

4-6. Effect of Zinc Supplementation on the Risk Factors of CVDs

**Animal Studies**

An animal study reported that zinc supplementation for 8 weeks suppressed lipid accumulation in aortic lesions and decreased atherosclerotic lesion size in New Zealand white rabbits fed with a high-cholesterol diet108). In addition, another study using atherogenic mice found that additional supplementation of zinc for 4 weeks suppressed the abnormality of the plasma lipid profile and the expression of inflammatory markers in aortic lesions in LDL-receptor-deficient mice109).

**Human Studies**

Regarding humans, a recent meta-analysis that analyzed a total of 20 randomized controlled trials including 1,141 subjects has shown that zinc supplementation decreased plasma levels of triglycerides, very low-density lipoprotein -cholesterol and total cholesterol as well as fasting plasma glucose and HbA1c levels in patients with metabolic disorders, including diabetic mellitus110). This study suggests that zinc supplementation can reduce the risk of atherosclerosis and CVDs. In contrast, previous meta-analysis including 32 clinical trials showed no effect of zinc on cardiometabolic risk factors, such as lipid profiles111). Although the reasons of these discrepancies remain unresolved, dose and duration of zinc supplementation may affect the effect of zinc on the risk of CVDs. The latest recent meta-analysis revealed that low-dose zinc supplementation (<25 mg/d) and long-duration (≥12 weeks) improved fasting blood glucose and serum lipids, including total cholesterol and LDL, more than high-dose (<25 mg/d) and short-duration (<12 weeks) interventions112). To determine the most effective dose and duration of zinc supplementation, further study seems to be necessary.

Conclusions

Collectively, the above findings indicate that zinc can regulate glucose and lipid metabolism as well as vascular function and that intracellular zinc dyshomeostasis is associated with the increased risk of diabetes and CVDs. Altered zinc transporter expression and its dysfunction are deeply involved in insulin resistance and impaired insulin secretion, leading to diabetes. Although the detailed role of impaired zinc homeostasis in vascular dysfunction remains unclear, recent emerging evidence suggests the significant role of zinc transporters in the regulation of endothelial and VSMC functions. To establish zinc replacement therapy as an effective strategy for the prevention and treatment of diabetes and CVDs, further understanding of the role of intracellular zinc homeostasis in the regulation of glucose metabolism and vascular function is necessary.

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Conflict of Interest

The author declares that there is no conflict of interest.
References

1) Fishman SL, Sonmez H, Basman C, Singh V and Poretzky L: The role of advanced glycation end-products in the development of coronary artery disease in patients with and without diabetes mellitus: a review. Mol Med, 2018; 24: 59

2) Gray SP and Jandeleit-Dahm K: The pathobiology of diabetic vascular complications--cardiovascular and kidney disease. J Mol Med (Berl), 2014; 92: 441-452

3) Iwakawa N, Tanaka A, Ishii H, Kataoka T, Niwa K, Hitora Y, Tashiro H, Mitsuda T, Kojima H, Hirayama K, Furusawa K, Yoshida R, Suzuki S and Murohara T: Impact of Diabetes Mellitus on the Aortic Wall Changes as Atherosclerosis Progresses: Aortic Dilatation and Calcification. J Atheroscler Thromb, 2020; 27: 509-515

4) King JC, Shames DM and Woodhouse LR: Zinc homeostasis in humans. J Nutr, 2000; 130: 1360S-1366S

5) Prasad AS: Discovery of human zinc deficiency: its impact on human health and disease. Adv Nutr, 2013; 4: 176-190

6) Fukunaka A and Fujitani Y: Role of Zinc Homeostasis in the Pathogenesis of Diabetes and Obesity. Int J Mol Sci, 2018; 19;

7) Choi S, Liu X and Pan Z: Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases. Acta Pharmacol Sin, 2018; 39: 1120-1132

8) Zalewski PD, Beltrame JF, Wawer AA, Abdo AI and Murgia C: Roles for endothelial zinc homeostasis in vascular physiology and coronary artery disease. Crit Rev Food Sci Nutr, 2019; 59: 3511-3525

9) Valko M, Jomova K, Rhodes CJ, Kuca K and Musilek K: Free radicals and their role in human disease. Arch Toxicol, 2016; 90: 1-37

10) Iwakawa N, Tanaka A, Ishii H, Kataoka T, Niwa K, Hitora Y, Tashiro H, Mitsuda T, Kojima H, Hirayama K, Furusawa K, Yoshida R, Suzuki S and Murohara T: Impact of Diabetes Mellitus on the Aortic Wall Changes as Atherosclerosis Progresses: Aortic Dilatation and Calcification. J Atheroscler Thromb, 2020; 27: 509-515

11) Zalewski PD, Millard SH, Forbes IJ, Kapaniris O, Slavotinseck A, Betts WH, Ward AD, Lincoln SF and Mahadevan I: Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. J Histochem Cytochem, 1994; 42: 877-884

12) Zalewski PD, Millard SH, Forbes IJ, Kapaniris O, Slavotinseck A, Betts WH, Ward AD, Lincoln SF and Mahadevan I: Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. J Histochem Cytochem, 1994; 42: 877-884

13) Foster MC, Leapman RD, Li MX and Atwater I: Elemental composition of secretory granules in pancreatic islets of Langerhans. Biophys J, 1993; 64: 525-532

14) Zalewski PD, Millard SH, Forbes IJ, Kapaniris O, Slavotinseck A, Betts WH, Ward AD, Lincoln SF and Mahadevan I: Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. J Histochem Cytochem, 1994; 42: 877-884

15) Foster MC, Leapman RD, Li MX and Atwater I: Elemental composition of secretory granules in pancreatic islets of Langerhans. Biophys J, 1993; 64: 525-532

16) Dodson EJ, Dodson GG, Hodgkin DC and Reynolds CD: Structural relationships in the two-zinc insulin hexamer. Can J Biochem, 1979; 57: 469-479

17) Dunn MF: Zinc-ligand interactions modulate assembly and stability of the insulin hexamer -- a review. Biometals, 2005; 18: 295-303

18) Scott DA and Fisher AM: The Insulin and the Zinc Content of Normal and Diabetic Pancreas. J Clin Invest, 1938; 17: 725-728

19) Boquist L and Lernmark A: Effects on the endocrine pancreas in Chinese hamsters fed zinc deficient diets. Acta Pathol Microbiol Scand, 1969; 76: 215-228

20) Huber AM and Gershoff SN: Effect of zinc deficiency in rats on insulin release from the pancreas. J Nutr, 1973; 103: 1739-1744

21) Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pechetsky AV, Pretinki M, Posner BI, Balding DJ, Meyre D, Polychronakos C and Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature, 2007; 445: 881-885

22) Fauchet S, Del Guerra S, Choquet H, D’Alcoo V, Groves CJ, Lapi R, McCarthy MI, Froguel P and Marchetti P: Meta-analysis and functional effects of the SLC30A8 rs13266634 polymorphism on isolated human pancreatic islets. Mol Genet Metab, 2010; 100: 77-82

23) Boesgaard TW, Zilinskaite J, Vanntinen M, Laaks M, Jansson PA, Memarstedt A, Smith U, Stefan N, Fritsche A, Haring H, Hribal M, Sesti G, Zobel DP, Pedersen O, Hansen T and Consortium E: The common SLC30A8 Arg325Trp variant is associated with reduced first-phase insulin release in 846 non-diabetic offspring of type 2 diabetes patients--the EUGENE2 study. Diabetologia, 2008; 51: 816-820

24) Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B, Kerr-Conte J, Van Lommel S, Grunwald D, Favier A and Seve M: In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion. J Cell Sci, 2006; 119: 4199-4206

25) Makami M, Fujitani Y, Hara A, Uchida T, Tamura Y, Takeno K, Kawaguchi M, Watanabe T, Ogihara T, Fukunaka A, Shimizu T, Mita T, Kanazawa A, Imaizumi MO, Abe T, Kiyonari H, Hoyo J, Sato G, Zobel D, Pedersen O, Hansen T and Consortium E: The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance. J Clin Invest, 2013; 123: 4513-4524

26) Wijesekara N, Dai FF, Hardy AB, Gigliow PR, Bhattacharjee A, Koshkin V, Chimienti F, Gaisano HY, Rutter GA and Wheeler MB: Beta cell-specific Znt8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia, 2010; 53: 1656-1668

27) Nicolson TJ, Bellomo EA, Wijesekara N, Loder MK,
Baldwin JM, Gyuikhandanyan AV, Koshkin V, Tarasov AI, Carzaniga R, Kronenberger K, Taneja TK, da Silva Xavier G, Libert S, Freguol P, Scharffmann R, Steetsuyk V, Ravassard P, Parker H, Gribble FM, Reimann F, Sladek R, Hughes SJ, Johnson PR, Masseboef M, Burcelin R, Baldwin SA, Liu M, Lara-Lemus R, Arvan P, Schuit FC, Wheeler MB, Chimienti F and Rutter GA: Insulin storage and glucose homeostasis in islets for the granule zinc transporter ZnT8 and studies of the type 2 diabetes-associated variants. Diabetes, 2009; 58: 2070-2083

28) Wong WP, Allen NB, Meyers MS, Link EO, Zhang X, MacRenaris KW and El Muayed M: Exploring the Association Between Demographics, SLC30A8 Genotype, and Human Islet Content of Zinc, Cadmium, Copper, Iron, Manganese and Nickel. Sci Rep, 2017; 7: 473

29) Li J, Bai S and Sheline CT: hZnT8 (Slc30a8) Transgenic Mice That Overexpress the R325W Polymorph Have Reduced Islet Zn2+ and Proinsulin Levels, Increased Glucose Tolerance After a High-Fat Diet, and Altered Levels of Pancreatic Zinc Binding Proteins. Diabetes, 2017; 66: 551-559

30) Smidt K, Jessen N, Petersen AB, Larsen A, Magnussen N, Jeppesen JB, Stoltenberg M, Culvenor JG, Tsatsanis A, Brock B, Schmitz O, Wogensen L, Bush AI and Rungby J: SLC30A3 responds to glucose- and zinc variations in beta-cells and is critical for insulin production and in vivo glucose-metabolism during beta-cell stress. PLoS One, 2009; 4: e5684

31) Smidt K, Larsen A, Bronden A, Sorensen KS, Nielsen JV, Praetorius J, Martensen PM and Rungby J: The zinc transporter ZNT3 co-localizes with insulin in INS-1E pancreatic beta cells and influences cell survival, insulin secretion capacity, and ZNT8 expression. Biometals, 2016; 29: 287-298

32) Huang L, Yan M and Kirschke CP: Over-expression of ZnT7 increases insulin synthesis and secretion in pancreatic beta-cells by promoting insulin gene transcription. Exp Cell Res, 2010; 316: 2630-2643

33) Wang X, Gao H, Wu W, Xie E, Yu Y, He X, Li J, Zheng W, Wang X, Cao X, Meng Z, Chen L, Min J and Wang F: The zinc transporter Slc39a5 controls glucose sensing and insulin secretion in pancreatic beta-cells via Sirt1- and Pgc-1alpha-mediated regulation of Glut2. Protein Cell, 2019; 10: 436-449

34) Cruz KJC, de Oliveira ARS, Morais JBS, Severo JS, Mendes PMV, de Sousa Melo SR, de Sousa GS and Marreiro DDN: Zinc and Insulin Resistance: Biochemical and Molecular Aspects. Biol Trace Elem Res, 2018; 186: 407-412

35) Bellomo E, Massarotti A, Hogstrand C and Maret W: Zinc ions modulate protein tyrosine phosphatase 1B activity. Metallomics, 2014; 6: 1229-1239

36) Vardatsikos G, Pandey NR and Srivastava AK: Insulinomimetic and anti-diabetic effects of zinc. J Inorg Biochem, 2013; 120: 8-17

37) Barthel A, Ostrakhovitch EA, Walter PL, Kampkotter A and Klotz LO: Stimulation of phosphoinositide 3-kinase/Akt signaling by copper and zinc ions: mechanisms and consequences. Arch Biochem Biophys, 2007; 463: 175-182

38) Wu Y, Lu H, Yang H, Li C, Sang Q, Liu X, Liu Y, Wang Y and Sun Z: Zinc stimulates glucose consumption by modulating the insulin signaling pathway in L6 myotubes: essential roles of Akt-GLUT4, GSK3beta and mTOR-S6K1. J Nutr Biochem, 2016; 34: 126-135

39) Ilouz R, Kaidanovich O, Gurwitz D and Eldar-Finkelman H: Inhibition of glycogen synthase kinase-3beta by bivalent zinc ions: insight into the insulin-mimetic action of zinc. Biochem Biophys Res Commun, 2002; 295: 102-106

40) Cameron AR, Anil S, Sutherland E, Harthill J and Rena G: Zinc-dependent effects of small molecules on the insulin-sensitive transcription factor FOXO1a and gluconeogenic genes. Metallomics, 2010; 2: 195-203

41) Oh KJ, Han HS, Kim MJ and Koo SH: CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis. BMB Rep, 2013; 46: 567-574

42) Huang L, Kirschke CP, Lay YA, Levy LB, Lamirande DE and Zhang PH: Znt7-null mice are more susceptible to diet-induced glucose intolerance and insulin resistance. J Biol Chem, 2012; 287: 33883-33896

43) Huang L, Tepaamorndech S, Kirschke CP, Newman JW, Keyes WR, Pedersen TL and Dumnill J: Aberrant fatty acid metabolism in skeletal muscle contributes to insulin resistance in zinc transporter 7 (znt7)-knockout mice. J Biol Chem, 2018; 293: 7549-7563

44) Myers SA, Nield A, Chew GS and Myers MA: The zinc transporter, Slc39a7 (Zip7) is implicated in glycaemic control in skeletal muscle cells. PLoS One, 2013; 8: e79316

45) Aydemir TB, Troche C, Kim MH and Cousins RJ: Hepatic ZIP14-mediated Zinc Transport Contributes to Endosomal Insulin Receptor Trafficking and Glucose Metabolism. J Biol Chem, 2016; 291: 23939-23951

46) Tepaamorndech S, Kirschke CP, Pedersen TL, Keyes WR, Newman JW and Huang L: Zinc transporter 7 deficiency affects lipid synthesis in adipocytes by inhibiting insulin-dependent Akt activation and glucose uptake. FEBS J, 2016; 283: 378-394

47) Fukunaka A, Fukada T, Bijn H, Suzuki L, Tsutsui T, Takamine Y, Bin BH, Yoshihara T, Ichinoseki-Sekine N, Naito H, Miyatsuka T, Takamiya S, Sasaki T, Inagaki T, Kitamura T, Kajimura S, Watada H and Fujitani Y: Zinc transporter ZIP13 suppresses beige adipocyte biogenesis and energy expenditure by regulating C/EBP-beta expression. PLoS Genet, 2017; 13: e1006950

48) Troche C, Aydemir TB and Cousins RJ: Zinc transporter Slc39a14 regulates inflammatory signaling associated with hypertrophic adiposity. Am J Physiol Endocrinol Metab, 2016; 310: E258-268

49) Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N and Kandhro GA: Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trace Elem Res, 2008; 122: 1-18

50) Hasanato RM: Trace elements in type 2 diabetes mellitus and their association with glycemic control. Afr Health Sci, 2020; 20: 287-293

51) Farooq DM, Alamri AF, Alwhahabi BK, Metwally AM and Kareem KA: The status of zinc in type 2 diabetic
patients and its association with glycemic control. J Family Community Med, 2020; 27: 29-36
52) Fernandez-Cao JC, Warthon-Medina M, Hall Moran V, Arija V, Doepking C and Lowe NM: Dietary zinc intake and whole blood zinc concentration in subjects with type 2 diabetes versus healthy subjects: A systematic review, meta-analysis and meta-regression. J Trace Elem Med Biol, 2018; 49: 241-251
53) Bandeira VDS, Pires LV, Hashimoto LL, Alencar LL, Almonds KGS, Lottenberg SA and Cozzolino SMF: Association of reduced zinc status with poor glycemic control in individuals with type 2 diabetes mellitus. J Trace Elem Med Biol, 2017; 44: 132-136
54) Forte G, Bocca B, Peruzzu A, Tolu F, Asara Y, Farace O, Oggiomo R and Maddeddu R: Blood metal concentrations in type 1 and type 2 diabetics. Biol Trace Elem Res, 2013; 156: 79-90
55) Sobczak AIS, Stefanowicz F, Pitt SJ, Ajjan RA and Stewart AJ: Total plasma magnesium, zinc, copper and selenium concentrations in type-I and type-II diabetes. Biometals, 2019; 32: 123-138
56) Samadi A, Isikhan SY, Tinkov AA, Lay I, Dosa MD, Skalny AV, Skalnaya MG, Chirumbolo S and Bjorklund G: Zinc, copper, and oxyester levels in patients with type 1 and type 2 diabetes mellitus. Clin Nutr, 2020; 39: 1849-1856
57) Southon S, Kechrid Z, Wright AJ and Fairweather-Tait SJ: Effect of reduced dietary zinc intake on carbohydrate and Zn metabolism in the genetically diabetic mouse (C57BL/KsJ db/db+). Br J Nutr, 1988; 60: 499-507
58) Sisnande T, Lima CK, da Silva DC, Beninatto TM, Alves NL, Amaral MJ, Miranda-Alves L and Lima L: Dietary zinc restriction promotes degeneration of the endocrine pancreas in mice. Biochim Biophys Acta Mol Basis Dis, 2020; 1866: 165675
59) Sun Q, van Dam RM, Willett WC and Hu FB: Prospective study of zinc intake and risk of type 2 diabetes in women. Diabetes Care, 2009; 32: 629-634
60) Shan Z, Bao W, Zhang Y, Wang X, Jin Y, Song Y, Yao P, Sun C, Hu FB and Liu L: Interactions between zinc transporter-8 gene (SLC30A8) and plasma zinc concentrations for impaired glucose regulation and type 2 diabetes. Diabetes, 2014; 63: 1796-1803
61) Valera P, Zavattari P, Sanna A, Pretti S, Marcella A, Mannu C, Tartaglione C, Bruno G and Songini M: Zinc and Other Metals Deficiencies and Risk of Type 1 Diabetes: An Ecological Study in the High Risk Sardinia Island. PLoS One, 2015; 10: e0141262
62) Samuelsson U, Oikarinen S, Hyoty H and Ludvigsson J: Low zinc in drinking water is associated with the risk of type 1 diabetes in children. Pediatr Diabetes, 2011; 12: 156-164
63) Haglund B, Ryckenberg K, Selinus O and Dahlquist G: Evidence of a relationship between childhood-onset type 1 diabetes and low groundwater concentration of zinc. Diabetes Care, 1996; 19: 873-875
64) Fernandez-Cao JC, Warthon-Medina M, V HM, Arija V, Doepking C, Serra-Majem L and Lowe NM: Zinc Intake and Status and Risk of Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis. Nutrients, 2019; 11:
80) Zou MH, Shi C and Cohen RA: Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest, 2002; 109: 817-826

81) Mendes Garrido Abregu F, Gobetto MN, Juriol LV, Caniﬁ c C, Elesgaray R, Tomat AL and Arranz C: Developmental programming of vascular dysfunction by prenatal and postnatal zinc deﬁ ciency in male and female rats. J Nutr Biochem, 2018; 56: 89-98

82) St Croix CM, Waterloos KJ, Dineley KE, Reynolds IJ, Levitan ES and Pitt BR: Nitric oxide-induced changes in intracellular zinc homeostasis are mediated by metallocion/ion. Am J Physiol Lung Cell Mol Physiol, 2002; 282: L185-192

83) St Croix CM, Stitt MS, Leelavanichkul K, Waterloos KJ, Pitt BR and Watkins SC: Nitric oxide-induced modulation of protein thiolate clusters as determined by spectral ﬂ uorescence resonance energy transfer in live endothelial cells. Free Radic Biol Med, 2004; 37: 785-792

84) Meeraarani P, Ramadas P, Toborek M, Bauer HC, Bauer H and Hennig B: Zinc protects against apoptosis of endothelial cells induced by linoleic acid and tumor necrosis factor alpha. Am J Clin Nutr, 2000; 71: 81-87

85) Connell P, Young VM, Toborek M, Cohen DA, Barve S, McClain CJ and Hennig B: Zinc attenuates tumor necrosis factor-mediated activation of transcription factors in endothelial cells. J Am Coll Nutr, 1997; 16: 411-417

86) Zhuang X, Pang X, Zhang W, Zhu J, Yang H and Qu W: Effects of zinc and manganese on advanced glycation end products (AGEs) formation and AGEs-mediated endothelial cell dysfunction. Life Sci, 2012; 90: 131-139

87) Alcantara EH, Shin MY, Feldmann J, Nixon GF, Beattie JH and Kwun IS: Long-term zinc deprivation accelerates rat vascular smooth muscle cell proliferation involving the down-regulation of JNK1/2 expression in MAPK signaling. Atherosclerosis, 2013; 228: 46-52

88) Allen-Redpath K, Ou O, Beattie JH, Kwun IS, Feldmann J and Nixon GF: Marginal dietary zinc deﬁ ciency in vivo induces vascular smooth muscle cell apoptosis in large arteries. Cardiovasc Res, 2013; 99: 525-534

89) Voelkl J, Tuffaha R, Luong TTD, Zickler D, Masyout J, Feger M, Verheyen N, Blaschke F, Kuro OM, Tomashitz A, Pilz S, Pasch A, Eckardt KU, Scherberich JE, Lang F, Pieske B and Alesutan I: Zinc Inhibits Phosphate-Induced Vascular Calcification through TNP-18203-Mediated Suppression of NF-kappaB. J Am Soc Nephrol, 2018; 29: 1636-1648

90) Abdo AI, Tran HB, Hodge S, Beltranme JF and Zalewski PD: Zinc Homeostasis Alters Zinc Transporter Protein Expression in Vascular Endothelial and Smooth Muscle Cells. Biol Trace Elem Res, 2020;

91) Giacconi R, Muti E, Malavolta M, Cardelli M, Pierpaoli S, Cipriano C, Costarelli L, Tesei S, Saba V and Mocchegiani E: A novel Zip2 Glx/Arg/Leu codon 2 polymorphism is associated with carotid artery disease in aging. Rejuvenation Res, 2008; 11: 297-300

92) Zhao L, Oliver E, Maratou K, Anaradis OD, Cotroneo E, Chen CN, Wang L, Arce C, Chabosseau PL, Ponsa-Cobas J, Frid MG, Muyon B, Webster Z, Aldashev A, Ferrer J, Rutter GA, Stenmark KR, Aitman TJ and Wilkins MR: The zinc transporter ZnT12 regulates the pulmonary vascular response to chronic hypoxia. Nature, 2015; 524: 356-360

93) Hershﬁ nkel M: The Zinc Sensing Receptor, ZnR/GPR39, in Health and Disease. Int J Mol Sci, 2018; 19: 525

94) Zhu D, Su Y, Zheng Y, Fu B, Tang L and Qin YX: Zinc regulates vascular endothelial cell activity through zinc-sensing receptor ZnR/GPR39. Am J Physiol Cell Physiol, 2018; 314: C404-C414

95) Koo SI and Lee CC: Compositional changes in plasma high-density lipoprotein particles in marginally zinc-deﬁ cient male rats. Am J Clin Nutr, 1988; 47: 120-127

96) Beattie JH, Gordon MJ, Duthie SJ, McNeil CJ, Horgan GW, Nixon GF, Feldmann J and Kwun IS: Suboptimal dietary zinc intake promotes vascular inﬂammation and atherogenesis in a mouse model of atherosclerosis. Mol Nutr Food Res, 2012; 56: 1097-1105

97) Kok FJ, Van Duijn CM, Hofman A, Van der Voet GB, De Wolff FA, Paays CH and Valkenburg HA: Serum copper and zinc and the risk of death from cancer and cardiovascular disease. Am J Epidemiol, 1988; 128: 352-359

98) Singh RB, Gupta UC, Mittal N, Niaz MA, Ghosh S and Rastogi V: Epidemiologic study of trace elements and magnesium on risk of coronary artery disease in rural and urban Indian populations. J Am Coll Nutr, 1997; 16: 62-67

99) Alissa EM, Bahjri SM, Ahmed WH, Al-Ama N and Ferns GA: Trace element status in Saudi patients with established atherosclerosis. J Trace Elem Med Biol, 2006; 20: 105-114

100) Kazemi-Bajestani SM, Ghayour-Mobarhan M, Ebrahimi M, Moohebati M, Esmaeili HA, Parizadeh MR, Aghacizadeh R and Ferns GA: Serum copper and zinc concentrations are lower in Iranian patients with angiographically deﬁ ned coronary artery disease than in subjects with a normal angiogram. J Trace Elem Med Biol, 2007; 21: 22-28
Nasir K, Sposito AC and Brazilian Study on Healthy Aging G: Low zinc levels is associated with increased inflammatory activity but not with atherosclerosis, arteriosclerosis or endothelial dysfunction among the very elderly. BBA Clin, 2014; 2: 1-6
104) Yesmin M, Hossain MS, Mia AR, Tabassum R, Parvin K, Akter R and Epsi EZ: Serum Zinc Status among Acute Myocardial Infarction Male Patients in Bangladesh. Mymensingh Med J, 2017; 26: 17-20
105) Huang L, Teng T, Zhao J, Bian B, Yao W, Yu X, Wang Z, Xu Z and Sun Y: The Relationship Between Serum Zinc Levels, Cardiac Markers and the Risk of Acute Myocardial Infarction by Zinc Quartiles. Heart Lung Circ, 2018; 27: 66-72
106) Chu A, Foster M and Samman S: Zinc Status and Risk of Cardiovascular Diseases and Type 2 Diabetes Mellitus-A Systematic Review of Prospective Cohort Studies. Nutrients, 2016; 8:
107) Liu B, Cai ZQ and Zhou YM: Deficient zinc levels and myocardial infarction: association between deficient zinc levels and myocardial infarction: a meta-analysis. Biol Trace Elem Res, 2015; 165: 41-50
108) Jenner A, Ren M, Rajendran R, Ning P, Huat BT, Watt F and Halliwell B: Zinc supplementation inhibits lipid peroxidation and the development of atherosclerosis in rabbits fed a high cholesterol diet. Free Radic Biol Med, 2007; 42: 559-566
109) Reiterer G, MacDonald R, Browning JD, Morrow J, Marveev SV, Daugherty A, Smart E, Toborek M and Hennig B: Zinc deficiency increases plasma lipids and atherosclerotic markers in LDL-receptor-deficient mice. J Nutr, 2005; 135: 2114-2118
110) Khazdouz M, Djalalinia S, Sarrafi Zadeh S, Hasani M, Shidfar F, Ataie-Jafari A, Asayesh H, Zarei M, Gorabi AM, Noroozi M and Qorbani M: Effects of Zinc Supplementation on Cardiometabolic Risk Factors: a Systematic Review and Meta-analysis of Randomized Controlled Trials. Biol Trace Elem Res, 2020; 195: 373-398
111) Foster M, Petocz P and Samman S: Effects of zinc on plasma lipoprotein cholesterol concentrations in humans: a meta-analysis of randomised controlled trials. Atherosclerosis, 2010; 210: 344-352
112) Pompano LM and Boy E: Effects of Dose and Duration of Zinc Interventions on Risk Factors for Type 2 Diabetes and Cardiovascular Disease: A Systematic Review and Meta-Analysis. Adv Nutr, 2021; 12: 141-160