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Review

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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[1-3]. 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[4]. 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[5]. Furthermore, zinc deficiency also increases the risk for diabetes and CVDs[6, 7]. 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[6, 8]. 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

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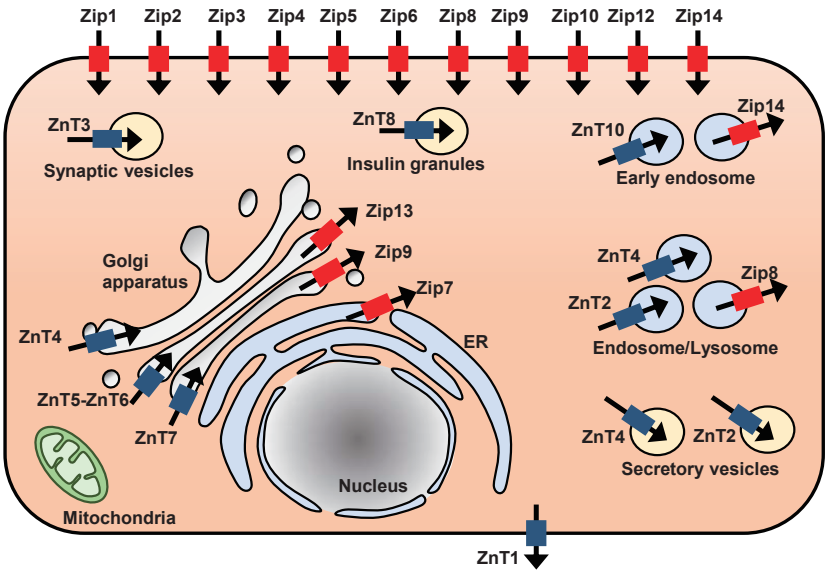

Fig. 1. Subcellular localization of a zinc transporter in mammalian cells (modified from reference (10)).
ER, endoplasmic reticulum

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 organelles 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[25]. This discrepancy appears to be explained by increasing insulin clearance in the liver of *ZnT8* KO mice[25]. Similar to *ZnT8* KO mice, *ZnT8 Arg325Trp* polymorphism in humans has also been shown to enhance insulin clearance[25]. 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, *in vitro* study using Min6 cells (mouse pancreatic β cell line) found that the overexpression of *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 *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 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

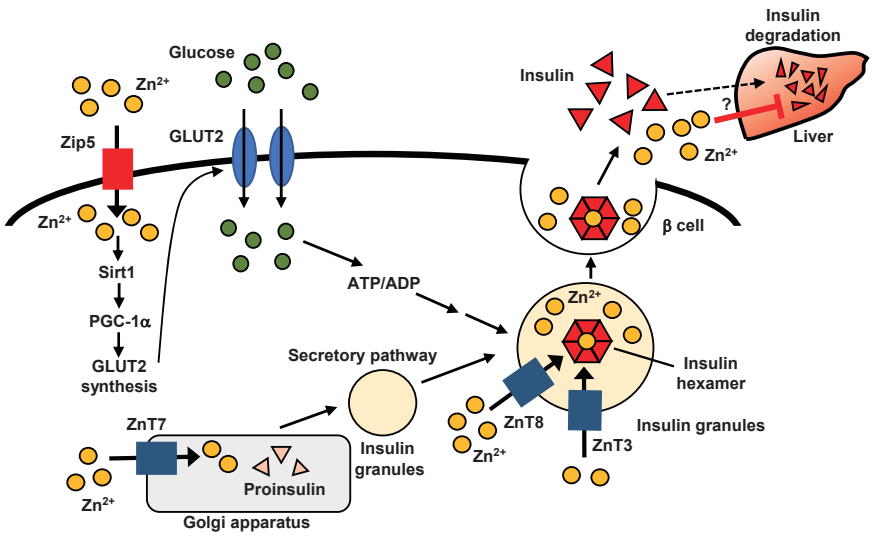

Fig. 2. Proposed mechanisms for the participation of zinc transporters (ZnT3, ZnT7, ZnT8, and Zip5) in the regulation of insulin processing, secretion, and clearance

Sirt1, sirtuin 1; PGC-1, peroxisome proliferator-activated receptor γ coactivator-1; GLUT2, glucose transporter 2

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* knockdown 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.

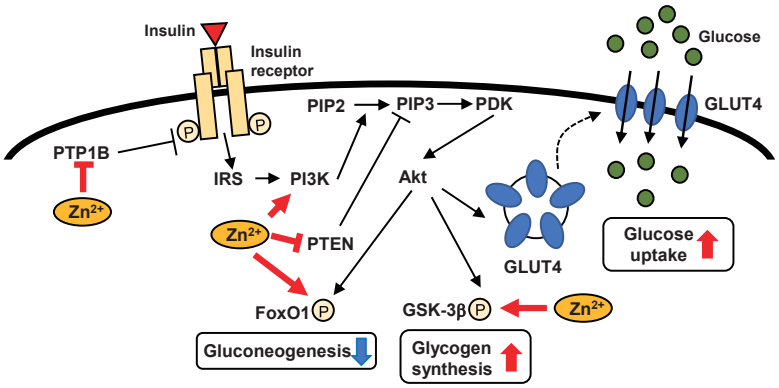

Fig. 3. Role of zinc in the regulation of insulin signaling (modified from reference (34))

PTPase, protein tyrosine phosphatase; IRS, Insulin receptor substrate; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4, 5-bisphosphate; PIP3, phosphatidylinositol 3, 4, 5-bisphosphate; PDK, phosphoinositide dependent kinase; PTEN, phosphatase and tensin homolog: Akt, protein kinase B; GSK-3β, glycogen synthase kinase 3β; FoxO1, forkhead box protein O1; GLUT4, glucose transporter 4

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)	Tepaamorndech 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)

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[45]. *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

endosomes in hepatocytes, resulting in the suppression of glucose uptake and glycogen synthesis.

In addition, ZnT7, Zip13, and Zip14 expressed in adipocytes have been suggested to be associated with insulin resistance by regulating adipogenesis and adipocyte function. In *ZnT7* KO mice, impaired fatty acid metabolism and insulin action were observed in subcutaneous fat[46]. Furthermore, knockdown of *ZnT7* decreased insulin-stimulated glucose uptake, resulting in decreased lipid accumulation in 3T3-L1 adipocytes, suggesting that ZnT7 positively regulates insulin signaling and lipid accumulation in adipocytes. Regarding Zip13, it has been reported that *Zip13* KO mice fed with a high-fat diet exhibited the enhanced energy expenditure through promoting beige adipocyte biogenesis, resulting in the amelioration of diet-induced insulin resistance[47]. It has also been reported that Zip14 regulates inflammatory signaling associated with hypertrophic adiposity[48].

Taken together, these findings suggest that mobilized zinc through zinc transporters regulates the insulin signaling and glucose metabolisms in insulin-sensitive organs, such as the muscle, liver, and adipose tissues, and that zinc dyshomeostasis is associated with the development of whole-body insulin resistance.

3-5. Blood Zinc Levels in Abnormal Glucose Metabolism

Several studies showed lower blood levels of zinc in patients with type 2 diabetes[49-51], 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 intake[52]. 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 zinc[53]. 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 subjects[54]. 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 subjects[55, 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 metabolism[57]. A study using a type 1 diabetic model mice also showed that dietary zinc restriction promoted degradation of the endocrine pancreas, resulting in augmented hyperglycemia[58]. 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 diabetes[59]. 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 diabetes[61]. Interestingly, lower zinc levels of drinking water have been shown to be associated with an increased risk of type 1 diabetes in children[62, 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, recent reports have shown that elevated serum/plasma zinc levels were associated with the increased risk of type 2 diabetes by 64%, despite the fact that moderately high dietary zinc intake could reduce the risk by 13%[64]. Therefore, the association between blood zinc levels and the development of diabetes remains controversial, and novel indicators may be needed to accurately assess zinc status in the body as an alternative to blood zinc levels.

3-7. Effect of Zinc Supplementation on Insulin Resistance and Diabetes

Animal Studies

Some animal studies have revealed the beneficial effect of zinc supplementation on metabolic abnormality in the pre-diabetic and diabetic states[65, 66]. Zinc supplementation for 14 weeks attenuated glucose and lipid metabolic abnormalities in mice fed with a high-fat diet[65]. In addition, dietary zinc supplementation for 6 weeks attenuated hyperglycemia and hyperinsulinemia in type 2 diabetic db/db mice[66].

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-α-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-κB (NF-κB) 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

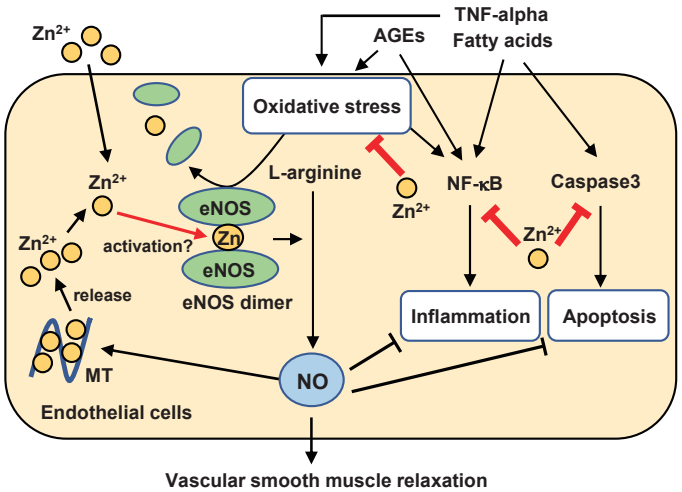

Fig. 4. Proposed mechanisms of the protective action of zinc in endothelial function

MT, metallothionein; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; TNF-α, tumor necrosis factor-alpha; NF-κB, nuclear factor-κB; AGEs, advanced glycation end products

vascular smooth muscle layer of blood vessels in rats[88]. 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-κB activity[89]. These findings suggest that zinc can prevent atherosclerosis and CVDs 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[90]. 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[90]. 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[91]. Regarding Zip12, it has been reported that the increased expression of Zip12 is observed in VSMCs in hypoxia-induced pulmonary arteries in rats[92]. 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[92]. 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[93]. 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 Gαq-Phospholipase C pathway[94]. 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 rats[95]. 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 diet[96], 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 reported[97-106]. A systematic review of a prospective cohort study showed that higher serum zinc levels are associated with a lower risk of CVDs[106]. Some studies have shown that lower zinc levels were inversely correlated with CVDs[98, 100, 101]. Furthermore, a meta-analysis of 13 case studies demonstrated that there are significant associations between zinc deficiency and the incidence of myocardial infarction[107]. 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 diet[108]. 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 mice[109].

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 mellitus[110]. 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 profiles[111]. 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) interventions[112]. 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.

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