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REVIEWS: CURRENT TOPICS

Zinc and diabetes — clinical links and molecular mechanisms

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Abstract

Zinc is an essential trace element crucial for the function of more than 300 enzymes and it is important for cellular processes like cell division and apoptosis. Hence, the concentration of zinc in the human body is tightly regulated and disturbances of zinc homeostasis have been associated with several diseases including diabetes mellitus, a disease characterized by high blood glucose concentrations as a consequence of decreased secretion or action of insulin. Zinc supplementation of animals and humans has been shown to ameliorate glycemic control in type 1 and 2 diabetes, the two major forms of diabetes mellitus, but the underlying molecular mechanisms have only slowly been elucidated. Zinc seems to exert insulin-like effects by supporting the signal transduction of insulin and by reducing the production of cytokines, which lead to beta-cell death during the inflammatory process in the pancreas in the course of the disease. Furthermore, zinc might play a role in the development of diabetes, since genetic polymorphisms in the gene of zinc transporter 8 and in metallothionein (MT)-encoding genes could be demonstrated to be associated with type 2 diabetes mellitus. The fact that antibodies against this zinc transporter have been detected in type 1 diabetic patients offers new diagnostic possibilities. This article reviews the influence of zinc on the diabetic state including the molecular mechanisms, the role of the zinc transporter 8 and MT for diabetes development and the resulting diagnostic and therapeutic options.

Keywords: Zinc; Diabetes mellitus; Insulin signaling; Cytokines; Zinc transporter; MT

1. Introduction

Since it was shown in 1934 that zinc is a component of insulin crystals [1], a relationship between zinc and diabetes mellitus has been proposed. From then on, numerous studies trying to elucidate the role of zinc in diabetes mellitus have been conducted with the aim of identifying new causal mechanisms and new therapeutical options.

The involvement of zinc in diabetes mellitus is not surprising because zinc is an essential trace element critical for the function of over 300 enzymes including members of all enzyme classes [2,3]. Hence, zinc plays a role in processes like DNA/RNA synthesis, cell division and apoptosis [4–7].

Only 2–4 g of zinc are present in the human body, and 12–16 μM can be normally measured in plasma, a mobile zinc pool that is required for the distribution of zinc [7–10]. Regarding these low amounts of zinc and the importance of this metal for enzyme function, it makes sense that the zinc concentration in the human body is tightly regulated by zinc transporters and zinc binding proteins like metallothionein (MT), which is capable of tightly binding zinc on the one hand and of releasing the metal dependent on the redox status on the other hand [11–13].

Disturbances of zinc homeostasis seem to be associated not only with diabetes, but also with several other diseases like cirrhosis of the liver [14–17], tumors [18,19], bowel disease [20,21], as well as with impaired function of the immune system [7,10,22–24]. The importance of an intact zinc homeostasis for the function of the immune system, a cell system consisting of frequently dividing cells, can be explained by the involvement of zinc in cell division [24]. Zinc has been shown to be necessary for physiological functioning of the innate and the adaptive immune system, and it is especially important for the development of T cells and their peripheral functions after maturation. High

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concentrations of zinc, however, lead to repression of T-cell functions, supporting the necessity of a tight regulation of the zinc concentration in the human body [24]. Moreover, zinc plays a role in humoral immunity regulating B-cell apoptosis as well as B-cell response to vaccination and it could be demonstrated that zinc modulates cytokine secretion [25–28].

The two forms of diabetes mellitus differ in their etiology. While type 1 diabetes is mainly an autoimmune process, only the minority of type 2 diabetes is based on an autoimmune process. The major immunological process is a destruction of pancreatic insulin-producing β cells, which is caused not only by T-cell-mediated cytotoxicity, but also by cytokine-induced cell death followed by the appearance of auto-antibodies [29–32].

Diabetes mellitus is characterized by chronic elevation of blood glucose concentration (hyperglycemia) as a consequence of decreased blood levels or decreased action of insulin [33], a hormone responsible for the regulation of plasma glucose concentration and glucose utilization [34,35].

In type 1 diabetes, the autoimmune destruction of β cells by the cellular and humoral immune system in the pancreatic islets of Langerhans leads to impaired insulin secretion and subsequently to hyperglycemia. This type of diabetes is characterized by the appearance of antigen-specific T cells and antibodies in peripheral blood which are directed against a variety of β-cell antigens including glutamic acid decarboxylase, tyrosine phosphatase IA-2, a zinc transporter and insulin. The onset of type 1 diabetes frequently occurs before 20 years of age, but disease manifestation is also common in adult patients. Exogenous administration of insulin is necessary to maintain glucose homeostasis and to prevent early and late diabetic complications [32,36]. In type 2 diabetes, comprising approximately 90% of the cases of diabetes mellitus, hyperglycemia is the consequence of a relative insulin deficiency and insulin resistance of various tissues including muscle and adipose tissue. While in early type 2 diabetes, insulin resistance and the resulting increased metabolic demand may be overcome by increased pancreatic insulin secretion, failure of β cells to maintain adequate insulin production and a decrease in β-cell mass are common in progressive disease, resulting in chronic hyperglycemia and loss of metabolic control [33,37,38]. Hyperinsulinemia is associated with down-regulation of insulin receptors, thus further contributing to the exhaustion of insulin production in β cells [39]. Overweight and obesity are significant risk factors for type 2 diabetes, which is increasing as a consequence of the Western lifestyle. Hence, diabetes is expected to become be an even greater health problem in the future deserving further attention [33,37].

Lifestyle modification including exercise, nutrition and behavioral changes is the cornerstone to prevent and treat type 2 diabetes. Oral antidiabetic medication — either as single agent or combination therapy — is frequently required to maintain metabolic control, as assessed by monitoring of glycated hemoglobin A_{1C} (HbA_{1C}) levels. Eventually, a significant proportion of patients with type 2 diabetes require the exogenous administration of insulin [40].

Type 1 diabetes as well as type 2 diabetes shows a genetic predisposition, although only type 1 diabetes is HLA dependent [32,33,36,40].

Complications arising from type 1 and type 2 diabetes mellitus comprise diabetic neuropathy, retinopathy, angiopathy and nephropathy, which are mainly due to the accumulation of advanced glycosylation end products [32,33,36,41,42]. The risk of diabetic complications increases with the duration of diabetes and the grade of hyperglycemia [33,36].

In order to prevent or delay the onset of such complications, tight control of fasting and postprandial blood glucose levels is a central aspect of diabetes treatment [32,33,36,40]. Current pharmacological therapy of diabetes is characterized by a variety of potential adverse events, including hypoglycemia, weight gain, elevation of liver enzymes, and heart failure. The development of the "ideal" antidiabetic agent, characterized by good efficiency, favourable safety profile and high clinical utility, including reasonable cost and ease of use, has therefore remained a central but elusive goal [40].

Zinc metabolism seems to be altered in diabetic patients as well as in diabetic animals, and zinc supplementation has been shown to exhibit beneficial effects in diabetic animals and humans, which indicates that this metal might qualify as a future therapeutic intervention in diabetes mellitus [43–50]. This hypothesis is supported by the identification of zinc transporter (ZnT)-8, a protein responsible for zinc regulation, possibly being part of the mechanisms leading to type 1 and type 2 diabetes [51–54] and by the proposed involvement of MT, another protein contributing to zinc homeostasis, in diabetes mellitus and its complications [55–60].

In this review, the altered metabolism of zinc in diabetes is characterized and the effects of zinc on the diabetic state are summarized taking into account the molecular mechanisms by which zinc interferes with the development and progression of diabetes and the genetic predisposition conferred by the genetic coding regions of ZnT-8 and MT.

2. Zinc metabolism and diabetes mellitus

Since zinc and diabetes mellitus have been linked by the discovery of zinc as part of the insulin complex [1], many studies examining zinc metabolism in diabetic animals and humans have been conducted.

The most consistent finding, including diabetic animal models like rats and mice as well as human diabetic patients, is the increased urinary excretion of zinc in diabetic subjects compared to controls [61–71]. In humans, there seem to be gender differences concerning zinc metabolism in diabetic subjects since diabetic females excreted more urinary zinc than diabetic males [62].

Until now, the cause of the hyperzincuria in diabetic animals and humans has not been clearly identified, but there are several studies indicating a correlation of increased zincuria with the urine volume, polyuria due to osmotic diuresis being one symptom of diabetes mellitus [65,66,72]. Blood glucose concentration, glycosuria and proteinuria are further factors associated with hyperzincuria [61,62,64,65,70]. This is consistent with the fact that polyuria has an influence on urinary zinc excretion since glycosuria due to high blood glucose concentrations leads to osmotic diuresis. These findings are supported by the observation that a decrease of the blood glucose level achieved by insulin treatment, for example, led to a reduction of zinc excreted via the urine [66].

Besides hyperzincuria, other possible mechanisms of zinc loss comprise enhanced intestinal secretion of zinc, which may be the case during inflammatory bowel disease due to exsudation of zinc–protein complexes into the intestinal lumen. Substances such as calcium, magnesium or other bivalent cations, phytate, fiber, phosphates and other chelating agents interfere with intestinal absorption of zinc and subsequently lead to an increase of intestinal zinc excretion [73,74]. Physiologically, zinc is also lost by sweating [73]. An interesting finding is the increased loss of zinc from the pancreas of mice lacking the genes for MT-I and MT-II. This zinc loss seems to be mediated through the plasma and could be explained by a higher turnover of zinc in the pancreas of mice lacking the MT genes providing an example of the pivotal role of MT for zinc homeostasis [75].

The frequently observed increased loss of urinary zinc in diabetic patients suggests that they may be zinc deficient if they do not compensate for the urinary loss by increased absorption of zinc or decreased intestinal excretion. There are indeed several studies measuring plasma or serum zinc concentration as indicator of the zinc status in diabetic animals and humans, and many of these studies show a decrease in plasma/serum zinc [50,63,64,67,70,72,76–79], but also the contrary could be shown [65,80–84].

A closer look at these findings reveals a certain dependence of the plasma or serum zinc status on the type of diabetes. Type 2 diabetes is usually associated with decreased plasma or serum zinc concentration, whereas in type 1 diabetes plasma or serum zinc is mostly elevated [63–65,76,79–84]. These elevated zinc levels in animal models of type 1 diabetes could be easily explained by the sudden destruction of pancreatic β cells by diabetogenic substances like alloxan or streptozotocin (STZ), thus causing the release of the zinc that was stored in β cells [80,81].

The interpretation of elevated plasma zinc levels in people with diabetes in spite of hyperzincuria is not quite as simple, but plasma zinc concentrations might be dependent on the duration of diabetes with higher plasma zinc at the beginning of type 1 diabetes, when the destruction of the β cells takes place, and with decreased zinc concentrations later on, when the hyperzincuria outweighs the zinc release from β cells. This hypothesis is supported by the finding of a negative correlation between duration of type 1 diabetes and plasma/serum zinc [65,85].

Furthermore, it has to be taken into consideration that neither plasma nor serum zinc is a reliable parameter of the actual zinc status of the body, since a reduced plasma or serum zinc might indicate a zinc deficiency, but a slight deficit of zinc that can already affect the immune system is not necessarily detectable by measuring plasma or serum zinc [7,86].

This difference between type 1 and type 2 diabetes could not be seen when measuring the zinc concentration in human leukocytes which was decreased independently of the type of diabetes mellitus [67,78,83]. But it has to be taken into account that in all these studies the plasma zinc concentration was decreased according to the intracellular zinc concentration of the leucocytes and that in two of these studies the type of diabetes mellitus was not defined [67,78]. Therefore these results should be interpreted with care.

The tendency of different plasma/serum zinc concentrations in type 1 and type 2 diabetic subjects is also reflected when examining the zinc status of different tissues. In mice and rats made diabetic by administration of STZ, a model of type 1 diabetes mellitus, the zinc concentration in liver and kidney was increased, possibly due to an increase in MT, which could be observed in STZ-diabetic mice [87–89]. In addition, the concentration of zinc in bones was elevated in STZ-diabetic rats [88,90], and in alloxan-diabetic rats, another animal model of type 1 diabetes mellitus, zinc was elevated in various tissues compared to controls [91].

According to the differences of the plasma or serum zinc levels in the different types of diabetes, genetically diabetic db/db mice (db=diabetic), a model of type 2 diabetes mellitus based on a splice mutant of the leptin-receptor leading to loss of function, showed a decrease of zinc in bones [63,92]. Takita et al. [71] examined Goto-Kakizaki (GK) rats as a model of type 2 diabetes observing lower zinc levels in kidneys, testis and fat, but higher zinc levels in spleen, pancreas and prostate, which might indicate a redistribution of zinc during the diabetic state.

Like the increased urinary zinc excretion, at least in models of type 1 diabetic animals, the altered concentration of serum zinc could be normalized by treatment of diabetes and reduction of the high blood glucose concentration. The elevated zinc concentration in tissues could be attenuated by insulin, which leads to the assumption that the blood glucose concentration may not only be responsible for the increased urinary zinc excretion, but also for the alterations of plasma or serum zinc concentration and that hormonal disturbances may lead to the changes in tissue zinc concentration [80,81,87]. Support for the involvement of glucose in the disturbances of the zinc status was provided by Davies et al. [93] who showed that plasma zinc in diabetic and control subjects dropped after oral administration of glucose as well as by Pedrosa et al. [85] and Al-Maroof et al. [50] who noticed a negative

correlation between serum zinc and duration of type 1 and type 2 diabetes.

These studies clearly demonstrate that zinc metabolism is adversely affected by diabetes mellitus. Thus, correction of the zinc status might be a therapeutical option.

3. Zinc and its beneficial effects on diabetes mellitus

Zinc may have beneficial effects when administered to diabetic patients considering that most of the patients, especially those with type 2 diabetes, show a zinc deficiency [50,64,67,70,72,76,78,79]. Insulin-like activity and insulin released from the pancreas were decreased in zinc-deficient rats compared to controls, whereas insulin synthesis was normal, indicating a role for zinc in maintaining activity and secretion of insulin [94].

This insulinomimetic activity of zinc could be demonstrated testing several zinc complexes which inhibited free fatty acid release after epinephrine stimulation of adipocytes *in vitro* and which lowered blood glucose in KKAy mice *in vivo* when administered intraperitoneally. A tendency to lower blood glucose was also observed when zinc complexes were administered orally [47,48,95–98]. In GK rats, serum glucose could be reduced by oral administration of zinc (ZnII) complexes [99]. Insulinomimetic activity of these complexes was strongly related to lipophilicity of the ligands, indicating that the complex has to reach intracellular space [97,100]. These observations suggest that zinc might have intracellular effects that mimic insulin action.

Examination of the effect of zinc supplementation making use of different animal models indicates possible positive effects of zinc on glycemic control in diabetic patients, which is usually assessed by measurement of the long-term-indicator for the glucose level, HbA_{1C}. Zinc supplementation before treatment of rats with alloxan or dithizone, which are diabetogenic drugs, prevented hyperglycemia and islet destruction [101,102]. Mice which received zinc-enriched drinking water from 1 week before STZ administration were protected from STZ diabetes assumingly as a result of the observed elevation of MT in pancreatic islets [103]. The antidiabetic and cytoprotective effect of MT was confirmed by transgenic overexpression of MT in β cells treated with STZ *in vitro* and *in vivo* resulting in less β-cell destruction, delay of diabetes onset and reduction of blood glucose [104]. Similarly, a reduction of blood glucose after STZ administration could be observed in rats pretreated with zinc subcutaneously, which led to elevated levels of MT in plasma and pancreas [105]. However, another study failed to confirm a protective effect of zinc-enriched drinking water on the development of alloxan-induced diabetes in mice. Interestingly, in this study, MT induced by zinc and alloxan could only be found in exocrine pancreatic cells [106]. These discrepancies might be due to differences in the study protocols concerning the time course of zinc administration prior to the induction of diabetes as well as to differences in animal strains.

Zinc has been shown to reduce insulin secretion of the β cells, which renders β cells less vulnerable to harmful factors [107] based on the finding that increased β-cell activity seems to predispose these cells to getting damaged by environmental factors [108,109]. This could be another mechanism leading to the prevention of type 1 diabetes in animals given alloxan or dithizone implying a role of zinc supplementation in prevention or delay of type 1 diabetes onset.

The finding of Huber and Gershoff [94] that pancreata from zinc-deficient rats released significantly less immunoreactive insulin and insulin-like activity compared to pair-fed controls seems contrary, but the time course of the changes of insulin secretion has not been examined and since the total amounts of insulin secreted are not reported by Sprietsma and Schuitemaker [107], a direct comparison of these two publications is difficult. In BioBreeding Wistar rats, which develop spontaneous type 1 diabetes, a high-zinc diet reduced the incidence of diabetes and ameliorated glycemic control. This result suggests that zinc is also able to prevent the onset of diabetes in genetically predisposed animals [110].

Even after manifestation of type 1 diabetes, zinc supplementation ameliorated the hyperglycemia and serum leptin levels in animals, indicating that zinc might also be useful as a therapeutic agent after manifestation of diabetes [43]. The fact that zinc as a component of prostate extract enhanced glucose utilization in STZ-diabetic rats and improved the diabetic condition indicates that other substances present in prostate extract like cyclo (His-Pro) and arachidonic acid might also contribute to the positive effects on the diabetic state [46].

Animal studies concerning type 2 diabetes are fewer, but the condition of type 2 diabetes in db/db mice was also improved by zinc leading to lower fasting glucose and lower fasting serum insulin while the pancreatic zinc concentration was raised [45]. GK rats receiving zinc-cyclo-(His-Pro) showed a stimulation of glucose metabolism [111]. These observations lead to the assumption that zinc may have beneficial effects on the diabetic condition in type 1 as well as in type 2 diabetes.

Studies of zinc supplementation conducted in humans showed that zinc supplementation was less favorable in type 1 diabetes [69,112] than in type 2 diabetes, where zinc supplementation led to a decrease of the insulin resistance index (homeostasis model assessment-estimated insulin resistance), a reduction of the typically elevated insulin concentration [49] and a reduction of HbA_{1C} [50]. Few studies failed to demonstrate a positive effect of zinc on type 2 diabetes [113] or its prevention [114], being possibly due to an inadequately high zinc dose in the first case [24,115]. In contrast, zinc supplementation in type 1 diabetes might even have negative effects on the HbA_{1C} level [69,112]. Taken together, these studies indicate some beneficial effects of zinc on the diabetic state, in humans especially concerning type 2 diabetes, but they also emphasize the necessity to carefully

consider the risk factors and to tightly control glycemic parameters and zinc status when supplementing zinc.

Not only glycemic control can be influenced by zinc, but also the feared diabetic complications. Zinc was shown to impair oxidative changes in the retina at the early onset stage of type 1 diabetic rats [116] and to reduce oxidative stress in type 1 diabetic patients with retinopathy [117]. There is growing evidence from studies on humans and animals that zinc has a protective effect concerning cardiomyopathy, probably by induction of MT, an assumption supported by the cardioprotective effect observed in transgenic mice overexpressing MT in cardiac muscle, which is suggested to be mediated by the antioxidative capacity of MT [55,56,118–120]. Furthermore, zinc deficiency is a risk factor of cardiomyopathy and myocardial infarction [121,122]. Concerning diabetic neuropathy, positive effects have been demonstrated with zinc supplementation leading to amelioration of nerve conduction velocity [123].

Since the pro-/antioxidative capacity of zinc is well known and since the antioxidant enzyme superoxide dismutase (SOD) is zinc dependent, the effects of zinc on oxidative stress in diabetes mellitus have been examined by several working groups. Zinc supplementation of diabetic patients ameliorated initially increased parameters of lipid peroxidation in both forms of diabetes mellitus [44,117,124]. SOD overexpression was shown to increase tolerance of pancreatic β cells to oxidative stress-induced diabetogenesis in mice protecting from the onset of type 1 diabetes induced by alloxan and STZ [125], whereas in humans, high levels of copper-zinc (CuZn)-SOD did not protect diabetic patients from reactive oxygen species [126]. The level of SOD in humans could not be raised by zinc supplementation, although plasma lipid peroxidation significantly decreased [44]. Thus, zinc seems to positively influence lipid peroxidation and oxidative stress, but whether this effect is mediated by CuZn-SOD remains questionable.

These empirical findings of zinc improving glycemic control and protecting from diabetic complications while reducing oxidative stress — the latter certainly representing one causal factor of these beneficial effects — raised the question as to which molecular mechanisms might be responsible for these zinc effects observed in diabetic animals and humans.

4. Underlying mechanisms of the beneficial effects of zinc on diabetes mellitus

Zinc supplementation resulted in amelioration of glycemic control of diabetic patients and animals indicating insulin-like effects [43,45–50,95–98], but the molecular mechanisms leading to the effects described have only slowly been elucidated.

The insulin-like effect of zinc ions was already examined in 1982 in isolated rat adipocytes [127]. It was found that 250–1000 μM zinc stimulated 3-*O*-methylglucose transport, glucose metabolism to carbon dioxide (CO_2), glyceride-fatty acid and glyceride glycerol, as well as the pentose phosphate cycle. As mechanism behind these observations, a selective inhibition of the glutathione reductase was assumed, leading to a decrease of glutathione and a stimulation of the pentose-phosphate cycle [127]. Glucose oxidation was enhanced by hydrogen peroxide (H_2O_2), which in turn was increased by zinc. Most of the insulin-like effects of zinc seem to be dependent on the enhancement of glucose transport, but there seem to be a few intracellular effects as well [127].

4.1. Zinc and its influence on insulin signaling

Some of the insulinomimetic effects of zinc can be explained by the influence of zinc on insulin signaling. One zinc-dependent molecule, insulin-responsive aminopeptidase (IRAP), which is expressed and characterized in fat and muscle as insulin target tissues [128], seems to be required for the maintenance of normal glucose transporter (GLUT) 4 levels in order to ensure glucose uptake into tissues [129] (Fig. 1). As response to insulin, IRAP, which is stored in intracellular membrane compartments, is translocated to the cell surface and this translocation, similar to the insulin-induced translocation of GLUT 4, is impaired in type 2 diabetes mellitus [146,147].

Ezaki [148] showed that zinc induces the translocation of GLUT to the plasma membrane, resulting in an increased uptake of glucose into tissue cells, thereby lowering the blood glucose level. Since an intact binding site of the insulin receptor was not required for the translocation of GLUT, binding of zinc to the binding site of the insulin receptor cannot be the trigger. In addition, zinc did not have any effect on the insulin receptor kinase activity; hence a postinsulin-receptor mechanism was proposed [148]. This mechanism was further characterized by Tang et al. [143] who provided evidence that zinc potentiates insulin-induced glucose transport and increases glucose transport into cells acting through the insulin signaling pathway (Fig. 1).

In brief, binding of the insulin molecule to the α subunits of the tetrameric insulin receptor, which consists of two α- and two β subunits, leads to stimulation of the kinase activity of the β subunit, to transphosphorylation of the β subunit and to a subsequent conformational change that further enhances kinase activity [35,130]. As a consequence, cellular proteins such as members of the insulin receptor substrate (IRS) family are phosphorylated, which in turn interact with signaling molecules consisting of Src-homology-2 (SH-2) domains like the p85 regulatory subunit of phosphoinositide-3-kinase [PI3K] [131], a molecule that is important for the understanding of the zinc effects on insulin signal transduction.

The activated PI3K promotes the phosphorylation of phosphoinositide-dependent kinase 1 (PDK1), a serine kinase that activates and phosphorylates the serine/threonine kinase Akt, also called protein kinase B (PKB) [132,133]. Akt, in turn, is sufficient to stimulate GLUT 4 translocation in adipocytes, thereby enhancing glucose uptake [134] and it

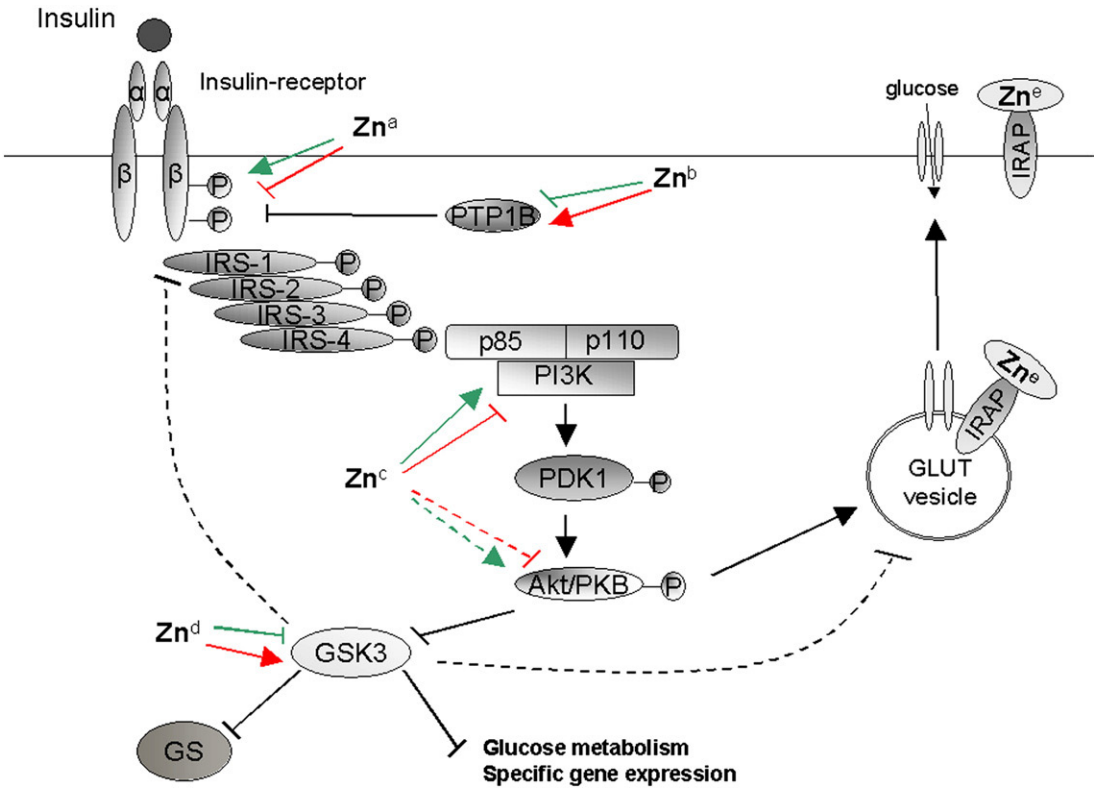

Fig. 1. Influence of zinc on the insulin signaling pathway. (⟶) Activation; (⟞) inhibition; (⇢) activation/inhibition of Akt by zinc/zinc deficiency might not be a direct effect of zinc on Akt but might be mediated by stimulation of PI3K; (– – ⟞) inhibition under certain conditions described in the review; green: insulinomimetic effects of zinc; red: effects of zinc deficiency leading to insulin resistance. After binding of insulin to the α subunits of the tetrameric insulin receptor, the kinase activity of the β subunit is stimulated, which results in transphosphorylation of the β subunit [35,130]. This induces phosphorylation of members of the IRS family and subsequent interaction with signaling molecules like the p85 subunit of the PI3K [131]. PI3K in turn triggers phosphorylation of PDK1, a serine kinase that activates Akt/PKB [132,133]. Akt leads to stimulation of GLUT 4 translocation in adipocytes and to inhibition of GSK-3, thereby allowing activation of glycogen synthase in adipocytes, translocation of GLUT to the cell surface and induction of glucose metabolism [35,134–141]. In addition, inhibition of GSK-3 results in enhanced protein synthesis and gene expression [35,142]. Zinc leads to tyrosine phosphorylation of the β subunit of the insulin-receptor [143[a]] and to inhibition of PTP1B which dephosphorylates the insulin receptor, thus increasing phosphorylation of the receptor [144[b]]. Akt is activated by zinc in a PI3K-dependent way [143[c]] and zinc inhibits GSK-3, just like insulin [145[d]]. Moreover, zinc plays a role in glucose transport since it is part of IRAP, a molecule probably required for maintenance of normal GLUT levels [129[e]]. Zn: zinc.

plays a role in transmission of the insulin signal by phosphorylation of serine residues and inhibition of glycogen synthase kinase 3 (GSK-3) [135–137,149]. GSK-3 is a constitutively active enzyme, consisting of two isoforms GSK-α and GSK-β, which are inactivated by inhibitory serine phosphorylation in response to insulin [150–152]. This inhibition leads to dephosphorylation and activation of glycogen synthase (GS) in adipocytes, an enzyme required for glycogen synthesis [138,153]. GSK-α seems to be important for hepatic glucose metabolism, whereas GSK-β is presumably responsible for the regulation of GS in skeletal muscle [154,155].

Inhibition of GSK-3 further enhanced basal glycogen synthase activity and insulin-stimulated glucose transport in insulin-resistant rat skeletal muscle possibly by increasing the presence of GLUT 4 at the cell surface after insulin stimulation [139–141]. It is unlikely that acute inhibition of GSK-3 plays a role in rapid stimulation of glucose transport by insulin in (non-insulin-resistant) muscle and fat cells, but sustained inhibition (6–24 h) caused an enhancement in basal glucose uptake in L6 myotubes, whereas insulin-stimulated glucose uptake remained unaffected [138] and a stimulation of both basal and insulin-regulated glucose uptake in human skeletal muscle cells [151].

An inhibitor of GSK-3 that is not an analog of adenosine triphosphate but which is substrate competitive even enhanced basal and insulin-stimulated glucose transport in primary mouse adipocytes after 1 h of incubation. This leads to the assumption that the mechanism of GSK-3 inhibition has a certain influence on the produced effects [156].

Mussmann et al. [157] showed that specific GSK-3 inhibitors increased β-cell replication rate in isolated rat islets and in a rat insulinoma cell line (INS cells) and that these inhibitors protected β cells against death induced by high concentrations of glucose and the saturated fatty acid palmitate.

Two negative feedback loops in this insulin signaling pathway are of interest. Additionally to tyrosine phosphorylation, both the insulin receptor and IRS proteins are also phosphorylated on serine residues, which may attenuate

signaling by decreasing insulin-stimulated tyrosine phosphorylation. This is mediated by PI3K, Akt, GSK-3 and mammalian target of rapamycin [35]. GSK-3 is capable of phosphorylating IRS-1, subsequently converting this molecule into an inhibitor of the insulin receptor tyrosine kinase activity *in vitro* and in insulin-resistant rat muscle after insulin stimulation [141,158]. A second mechanism negatively influencing insulin signaling is the rapid dephosphorylation of the insulin receptor and its substrates by protein tyrosine phosphatase 1B (PTP1B) [35].

The translocation of GLUT initiated by insulin is believed to be regulated through the insulin signaling pathway including molecules like IRS, PI3K, protein kinase C isoforms, and Akt or PKB [159–163], and like insulin, zinc enhances glucose uptake into fibroblasts and adipocytes, which suggests an involvement of zinc in this pathway. Examining the effects of zinc on the insulin signal transduction, it was observed that zinc leads to tyrosine phosphorylation of the β subunit of the insulin receptor, but to a lower extent compared to insulin, and that IRS does not seem to play a role in enhancing glucose uptake as a response to zinc stimulus [143]. According to this model, which proposes an activation of PI3K without involvement of IRS, zinc may induce the production of H_2O_2 by epididymal cells, which in turn causes the activation of focal adhesion kinase (FAK) and FAK can finally activate the PI3K-Akt pathway [143].

Support for the involvement of zinc in phosphorylation of the insulin receptor was provided by Haase and Maret [144] who identified PTP1B as a sensitive target of zinc ions and an important regulator of the phosphorylation state of the insulin receptor. Inhibition of PTP1B by zinc ions, which might be released from MT, leads to an increased phosphorylation status of the insulin receptor triggering the post-receptor events. Considering that oxidative stress leads to a release of zinc from MT and to cellular zinc depletion, this condition as well as zinc deficiency due to decreased absorption, increased excretion or increased requirements could possibly lead to diabetes mellitus [144].

Furthermore, zinc increased phosphorylation of serine residues and therefore activation of Akt in preadipocytes and adipocytes thereby enhancing GLUT translocation [134]. This effect could be blocked by wortmannin, an inhibitor of PI3K, underlining the importance of PI3K for the activation of Akt by zinc [143].

A further target molecule of zinc belonging to the insulin signaling pathway is GSK-3β [145], which has been shown to be elevated in the muscle of type 2 diabetic patients concerning its protein level and its activity, thus contributing to impaired glycogen synthase activity and skeletal muscle insulin resistance in type 2 diabetic patients. Considering the possibility of serine phosphorylation and inhibition of IRS-1 by GSK-3, the effects of elevated GSK-3 might be even broader affecting upstream events of the insulin signaling [158,164]. GSK-3 is uncompetitively inhibited by zinc thereby leading to increased glucose uptake [145]. This is consistent with the observation that zinc phosphorylates and activates Akt, which in turn inhibits GSK-3β. Consequently, all the functions of GSK-3 listed above would be impaired by zinc, resulting not only in enhanced glucose transport in insulin resistant cells, but also in increased glycogen synthesis by activation of glycogen synthase and in reduction of β-cell death as well as in augmented β-cell replication.

Taken together, zinc affects the insulin signaling pathway at several levels, inducing phosphorylation of the β subunit of the insulin receptor as well as of Akt and leading to inhibition of GSK-3β probably as a consequence of Akt phosphorylation, thereby exhibiting insulin-like effects. This suggests that zinc might be a therapeutical option in the treatment of diabetes mellitus or insulin resistance.

In support of the possible antidiabetic effects of zinc as an inhibitor of GSK-3, it was demonstrated in animal models that specific inhibitors of GSK-3 administered orally or subcutaneously rapidly lowered blood glucose levels and improved glucose disposal, insulin responses and insulin sensitivity after oral, intraperitoneal or intravenous glucose challenges in Zucker diabetic fatty rats and db/db mice, both models of type 2 diabetes mellitus, or insulin-resistant C57BL/6J mice [139,140,156].

4.2. Zinc and its effects on the cytokine-mediated destruction of β cells

Zinc does not only show insulin-like effects by interacting with molecules involved in insulin signal transduction, but it also protects insulin-producing β cells from death thereby ensuring a higher plasma insulin level. In type 1 diabetes, but also in type 2 diabetes, β-cell loss has been frequently observed due to an immune-mediated destruction of β cells with proinflammatory cytokines playing a major role [29–31].

Interleukin (IL)-1β, a mainly macrophage-derived cytokine, was demonstrated to be involved in the inhibition of glucose-stimulated insulin release and of proinsulin conversion [165,166] as well as in islet cell destruction in type 1 and type 2 diabetes [29,165–167]. These effects seem to be mediated by the activation of the transcription factor nuclear factor kappa B (NFκB) and subsequent nitric oxide (NO) production by inducible NO synthase (iNOS) [29,30,165,167–169] as well as by β-cell expression of Fas [29,30] (Fig. 2). NO appears to be less important as mediator of cytokine-induced pancreatic β-cell death in humans than in animals [29,30,165].

Nuclear factor-kappaB (NFκB) may have proapoptotic and antiapoptotic functions depending on the cytokine milieu to which the β cell is exposed and it leads to apoptosis after activation by interleukin-1 (IL-1) [166,169]. In human islets, IL-1β is usually not sufficient to induce β-cell apoptosis and additional cytokines like interferon (IFN)-γ and tumor necrosis factor (TNF)-α are required, which potentiate effects of IL-1β by influencing IL-1 signal transduction [29,30]. IL-1β can possibly be secreted by pancreatic β cells and can be induced by high glucose concentrations in cultured human islets [30,31,178], but Cnop et al. [166] did not observe IL-1β expression in β cells of a type 1 diabetic rat.

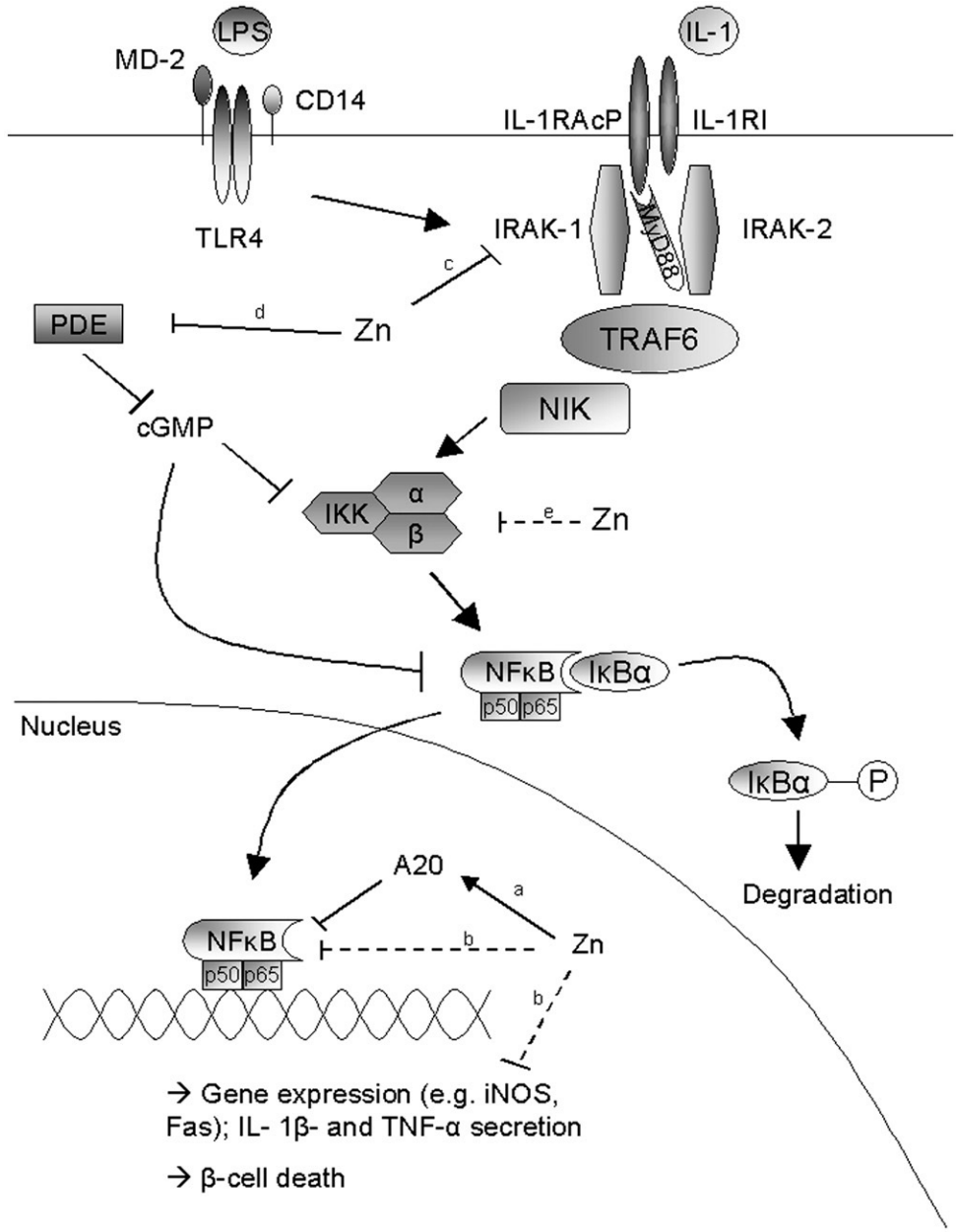

Fig. 2. Influence of zinc on TLR-/IL-1 signaling. (⟶) Activation; (⟞) inhibition; (– – ⟞) inhibition might be mediated by zinc acting upon upstream signaling events. The TLR-4 complex that is necessary for the recognition of LPS consists of a TLR4 homodimer combined with MD-2 and CD14, all binding to LPS. The following signaling cascade of the TLR receptor is similar to the signaling of the IL-1 receptor type I (IL-1RI) [170]. For IL-1 signaling, the association of IL-1RI with IL-1 receptor accessory protein (IL-1RAcP) is required. IL-1RAcP leads to recruitment of IRAK1, which in turn associates with TRAF6. TRAF6 is crucial for NFκB activation and translocation to the nucleus initiated by IL-1 since it induces IκB hyperphosphorylation by IKKα and IKKβ with NIK serving as a mediator activating IKK. Hyperphosphorylated IκB is degraded and NFκB is released. NFκB activates genes responsible for cytokine production, nitric monoxide synthesis and Fas expression, possibly inducing β-cell death [29,171,172]. IRAK2 associates with IL-1RI and it also activates NFκB in a TRAF-6-dependent way. MyD88 connects IL-1RAcP and IRAK2, binding to IRAK2 via its death domain [171]. Zinc has been shown to inhibit NFκB activation and to induce mRNA of A20, a protein negatively regulating NFκB [27[a],173[b]]. Moreover, zinc leads to inhibition of IRAK thereby inhibiting further signal transduction [174[c],175[c]] and to inhibition of PDE with subsequent cGMP-induced reduction of IKK and NFκB activity [28[d],176[d]]. In vitro, zinc also directly impaired LPS-induced IKK activity [177[c]]. NIK: NFκB-inducing kinase; TLR: Toll-like receptor; TRAF6: TNF receptor-associated factor-6.

IL-1β seems to have a bimodal function showing antidiabetic effects after a short exposure, especially when using low concentrations, and prodiabetic effects after a prolonged exposure, which could be the case in the development of diabetes mellitus [165,167,179,180].

Taking into account that in elderly individuals levels of proinflammatory cytokines are usually elevated, a process also known as “inflammaging” [181,182], the increasing risk of the development of type 2 diabetes with age [33] can be partly explained.

The destructive and diabetogenic role of IL-1β is further emphasized by the observation that blocking IL-1 signaling by IL-1 receptor antagonist (IL-1Ra) improved β-cell secretory function in type 2 diabetic patients and prevented destruction of cultured human β cells and diabetes in C57BL/6J mice fed high-fat/high-sucrose diet improving glucose tolerance and insulin secretion [167,178,183]. Support for the idea of IL-1 being involved in β-cell destruction was also provided by the finding that inhibition of NFκB, a molecule activated in the course of IL-1 signaling, conferred resistance to β-cell apoptosis in rodent and human islet cells [30,166].

IL-1 and NFκB are targets of zinc probably allowing the metal to interfere with the development of diabetes (Fig. 2). Although zinc stimulated peripheral blood mononuclear cells (PBMC) in a dose-dependent manner to release IL-1, IL-6, TNF-α and IFN-γ [25,184–187], and although it enhanced lipopolysaccharide (LPS)-induced secretion of IL-1β and TNF-α in PBMC and in whole-blood culture [188], the metal has also been shown to reduce LPS-induced IL-1β mRNA expression and release in Mono Mac cells and PBMC when pyrithione, an ionophore, was added [28]. Additionally, secretion of IL-1β was decreased in LPS-stimulated PBMC isolated from zinc-supplemented subjects [27]. In line with these findings are the observations of Kahmann et al. [86], who showed that, after zinc supplementation of healthy elderly subjects, the basal IL-6, IL-8 and IL-1β release of PBMC decreased, reaching statistical significance for IL-6 and IL-8. Similarly, Prasad et al. [189] demonstrated that after zinc supplementation of elderly subjects, *ex vivo* production of TNF-α, but not IL-1, significantly decreased.

These results suggest the controversial effects of zinc on cytokine production, which might be dependent on the zinc doses used or the time of zinc exposure. Furthermore, *in vivo* zinc administration might result in a different outcome concerning cytokine production compared to *in vitro* experiments. *In vitro*, administration of zinc up to 500 μM seems to stimulate cytokine production, whereas the combination of zinc and the ionophore pyrithione rather seems to decrease the secretion of proinflammatory cytokines [25,28,186] leading to the conclusion that high concentrations of intracellular zinc exert an inhibitory effect on cytokine production, whereas low concentrations of intracellular zinc promote cytokine production.

Zinc restriction led to increased IL-1β secretion in HL-60 cells, a human malignant monocyte-macrophage cell line, after 6 h of treatment with phorbol myristate acetate [26] and to an increased production of IL-1β by PBMC, which could be corrected by zinc supplementation [190]. In elderly persons with reduced plasma zinc, cells producing IL-1β and TNF-α were increased compared to younger subjects as well as the levels of the secreted cytokines [189]. These results suggest that zinc may play a role in reducing levels of IL-1β and therefore the incidence of β-cell destruction in type 1 and type 2 diabetes [29,165–167] and the effects of inflammaging on the development of type 2 diabetes [33,182].

Analyzing the molecular mechanisms that could be responsible for these observations, it was found that zinc inhibits cyclic nucleotide phosphodiesterases (PDE) subsequently leading to an increase in cyclic guanosine monophosphate (cGMP), which in turn mediates the inhibitory effect of zinc on TNF-α and IL-1β release by suppressing LPS-induced activation of inhibitory kappa B (IκB) kinase β (IKKβ) and NFκB [28,176] (Fig. 2).

NFκB is furthermore affected by zinc insofar as zinc supplementation led to (i) decreased TNF-α-induced NFκB activity in PBMC *ex vivo* [27], (ii) inhibition of NFκB activity and (iii) reduced expression of iNOS in pancreatic cells from CD1 mice made diabetic by alloxan or STZ [173]. Zinc was shown to interfere with the activation of NFκB by inhibiting IκB kinase (IKK) activity in LPS-stimulated RAW 264.7 cells which are mouse macrophages [177]. However, zinc deficiency can also result in reduced action of NFκB with impairment of the nuclear translocation of NFκB and of IκB phosphorylation in HUT-78 cells, a human T-helper(0) malignant lymphoblastoid cell line [191,192]. Controversial effects of zinc on NFκB were also observed by Schott-Ohly et al. [193] who found that after administration of zinc-enriched drinking water to NOD mice, which develop diabetes genetically, and to C57BL/6 mice made diabetic by multiple low doses of STZ, activation of NFκB and activator protein-1 was induced in the NOD mice and reduced in the STZ-diabetic C57BL/6 mice. This might indicate a certain dependence of the effect of zinc on NFκB on the mechanism of β-cell destruction [193].

Another molecular mechanism in addition to the PDE and IKK inhibition by which zinc exerts its inhibiting influence on NFκB might be the induction of mRNA of A20, a zinc-finger protein inhibiting NFκB activation, in HL-60 cells [27] (Fig. 2). Furthermore, the IL-1-induced NFκB activation could be suppressed by the zinc-dependent inhibition of the IL-1 type 1 receptor-associated kinase (IRAK) at high zinc levels (100 μM) [174], a concentration that probably cannot be safely achieved by zinc supplementation, but it could be demonstrated that the activation of the T-cell line EL4 6.1 through IL-1β was already decreased using a zinc concentration of 15 μM, which lies in the range of the physiological plasma zinc [7–10,175].

Nevertheless, these results suggest that zinc might exert protective effects on the diabetic state by inhibiting the cytokine-induced β-cell death via suppression of IL-1β secretion from monocytes/macrophages and suppression of NFκB activation.

5. The role of MT and the zinc transporter ZnT-8 in diabetes mellitus

Zinc homeostasis is maintained and regulated by zinc transport proteins and zinc binding proteins like MT with its

isoforms MT1–MT4 [11,13,194,195]. Considering that MT displays antioxidant capacities [120,196] and that it seems capable of preventing chemically induced diabetes in animals as well as the development of cardiomyopathy as discussed earlier, it is not surprising that polymorphisms in the genes of different isoforms of MT seem to play a role in diabetes mellitus.

One polymorphism in the gene of MT2A, characterized by an A/G transition, rs1610216, was shown to be associated with type 2 diabetes and atherosclerosis since the A allele was detected more frequently among Italian type 2 diabetic atherosclerotic patients compared to healthy controls [197].

Furthermore, the AA genotype in these patients was related to lower circulating zinc and higher glucose and HbA_{1C} values compared to patients carrying the AG genotype, indicating a greater insulin resistance, whereas there were no such differences in controls. Consistent with this finding is the higher rate of ischemic cardiomyopathy observed in type 2 diabetic atherosclerotic patients carrying the AA genotype compared to their AG counterparts leading to the conclusion that this single-nucleotide polymorphism (SNP) might predispose to type 2 diabetes and its cardiovascular complications [197].

Recently, an association between the SNP rs11640851, which is characterized by an A/C transition resulting in the amino acid substitution aspartate (Asp)→threonine (Thr) on Position 27 in the gene of MT1A, and type 2 diabetes has been demonstrated in an Italian population [59]. The C allele was significantly more frequent in type 2 diabetic patients with and without cardiovascular disease compared to controls. C+ carriers among the type 2 diabetic patients with cardiomyopathy displayed higher glycemia and HbA_{1C}, higher MT levels and a reduced intracellular zinc release compared to C− carriers [59]. Therefore, the SNP rs11640851 also seems to play a role in the pathogenesis or progression of type 2 diabetes including diabetic complications pointing out possible disturbances of zinc homeostasis.

For the second SNP in the gene of MT1A analysed in this study, rs8052394 [A/G transition; lysine (Lys)→arginine (Arg) on Position 51], no significantly different genotypic distributions could be observed [59].

However, this SNP has also been examined in a Chinese population and the G allele was shown to be associated with type 2 diabetes, indicating population differences concerning the distribution of SNPs [60]. Additionally, diabetic GG and GA carriers displayed a significant decrease in serum-SOD activity compared to diabetic AA carriers, indicating a higher rate of oxidative stress in diabetic carriers of the G allele, which might trigger the development of diabetic complications. The occurrence of diabetic neuropathy was associated with two further SNPs, rs11076161 and rs10636, in the genes of MT1A and MT2A, respectively. Finally, obesity being a major risk factor for the development of type 2 diabetes, the C allele of the SNP rs8052334 in MT1B was significantly associated with obesity when taking type 2 diabetic patients and controls together. In type 2 diabetic patients, the SNPs rs8052334 and rs10636 were shown to be significantly associated with hyperlipidemia, and the SNP rs964372 in the MT1B gene was significantly associated with increased serum triglyceride, indicating a possible predisposition to a disturbed fat metabolism [60].

These analyses emphasize the role of MT in the development of type 2 diabetes and its complications by influencing glycemic control, oxidative stress, zinc status and fat metabolism. The assumed involvement of MT in the pathogenesis of diabetes mellitus is further pointed out by the observation that MT-I and MT-II null mice showed a greater food intake, heavier body weight and higher plasma leptin levels, although liver zinc and plasma glucose were normal thereby developing a predisposition to type 2 diabetes [58]. Furthermore, levels of MT-I and MT-II in the plasma and skeletal muscle of type 2 diabetic patients were found to be significantly lower than those of controls, whereas markers of oxidative stress such as malondialdehyde and 8-oxoguanin were elevated in tissues of type 2 diabetics. The increase of MT in skeletal muscle after exercise as observed in controls could not be seen in diabetic patients, resulting in an even greater rise of tissue oxidative stress, supporting the idea of the involvement of oxidative stress and the dysregulation of MT in the pathogenesis of diabetes mellitus [57].

To sum up, MT may prevent or at least delay the onset of diabetes and the development of diabetic complications such as cardiomyopathy, whereas certain polymorphisms might predispose to type 2 diabetes mellitus, which could be mediated by a decreased function of MT or even by a reduction in the amount of protein as observed in type 2 diabetes [57].

Concerning the zinc transport proteins, two families have been identified so far. Import of zinc into the cytosol from either the extracellular space or from intracellular vesicles is mediated through the Zrt- and Irt-like proteins (ZIP) or solute-like carrier (SLC) 39 family, which comprises 14 members, ZIP1 to ZIP14.

The zinc-transporter (ZnT) proteins that belong to the SLC30 or cation diffusion facilitator family are responsible for zinc export from the cytosol into intracellular compartments like vesicles or the Golgi complex on the one hand and the extracellular space on the other hand. Ten ZnT transporters have been characterized as of the present (ZnT-1 to ZnT-10) [198–200].

ZnT-8 was identified exclusively in pancreatic β cells, where it could be visualized in intracellular insulin-containing secretory vesicles using a fluorescent fusion protein [51,52], but recently, the transporter could also be detected in human PBMC [11]. In order to ensure proper storage and maturation of insulin in its secretory vesicles, zinc is needed for structural stability of the storage form of insulin, a solid hexamer requiring two zinc ions [39]. The transport of zinc into secretory vesicles seems to be mainly accomplished by ZnT-8, indicating a possible role of this zinc transporter in the development of diabetes mellitus [51].

Recently, the field of research concerning zinc and diabetes was extended to the genomic level. In 2007, several genome-wide association studies analyzing the genetic background of diabetes mellitus by genotyping SNPs in a set of diabetic patients and a set of controls were conducted. A nonsynonymous SNP in SLC30A8 (the gene of ZnT8), rs13266634 [C/T transition; arginine (Arg) (325)→tryptophan (Trp) (325)], has frequently been shown to be associated with type 2 diabetes (Table 1).

The major allele 'C' of SLC30A8-SNP is the risk allele conferring a certain predisposition to type 2 diabetes. It does not have to be this SNP itself which is responsible for the association with diabetes, but genome-wide association studies assume that each genotyped SNP is a marker for the genomic region in linkage disequilibrium with this SNP. In the case of rs13266634, it is tempting to focus on the ZnT-8 transporter considering its localization and function, especially since the expression of ZnT-8 in human leukocytes strongly varied interindividually in contrast to the other ZnT, which showed a constant expression examining different individuals [11].

Regarding Table 1, it is obvious that the association between rs13266634 and type 2 diabetes cannot be shown in every study, which may be due to differences in sample sizes as well as to differences in population structure. Populations chosen from European countries consistently show the link between rs13266634 and type 2 diabetes mellitus, except for subpopulations from Denmark and the Netherlands examined by Steinthorsdottir et al. [211]. But when combining all study populations with European ancestry, the association between type 2 diabetes and rs13266634 in SLC30A8 is nevertheless highly significant [211].

For Japanese study populations, the results are less clear, including a study that proposes a nominal significance [206] and another which does not display a statistically significant association between type 2 diabetes and SLC30A8 [203]. In one study conducted in Japan, another SNP in the gene SLC30A8 was used, because rs13266634 deviated from the Hardy–Weinberg equilibrium [216]. This indicates that, in Japan, the distribution of the alleles of this SNP differs from the distribution in Europe, possibly explaining the difficulties in demonstrating the association which was shown in European populations. Similarly, in Asians, African Americans, Israelis, Moroccans and a population from West Africa, no association could be detected [201,207,210,211].

Given the association between rs13266634 in the European population strongly indicating an involvement of ZnT-8 in the development of type 2 diabetes mellitus, the

Table 1
Association between the polymorphism rs13266634 in SLC30A8 and type 2 diabetes mellitus

Study	Number of cases	Number of controls	Population	*P* value	Odds ratio (95% CI)
Cauchi et al. [201]	937	1000	France	.03	0.76 (0.59–0.97)[a]
	504	753	Austria	.01	0.76 (0.61–0.94)[a]
	577	552	Israel	No association	
	521	423	Morocco	No association	
DGI [202]	6529	7252	Finland/Sweden/ Poland/USA	.047	1.07 (1.0–1.16)
Furukawa et al. [203]	405	340	Japan	No association	
FUSION/Scott et al. [204]	2376	2432	Finland	.00005	1.18 (1.09–1.29)
Hertel et al. [205]	1638	1858	Norway	.00039	1.2 (1.09–1.33)
Horikoshi et al. [206]	864	864	Japan	Nominal association	
Lewis et al. [207]	993	1054	African Americans	No association	
Ng et al. [208]	3041	3678	Asia	.00065	1.13 (1.05–1.21)
Omori et al. [209]	1630	1064	Japan	.0073	1.225 (1.056–1.420)
Sanghera et al. [210]	532	386	Asia/India	No association	
Sladek et al. [53]	2617	2894	France	.000000061	Homozygous: 1.53±0.31 Heterozygous: 1.18±0.25
Steinthorsdottir et al. [211]	1399	5275	Iceland	.0006	1.19 (1.08–1.31)
	263	597	Denmark	No association	
	1359	4825	Denmark	No association	
	447	950	Philadelphia[b]	.000015	1.51 (1.25–1.81)
	368	915	The Netherlands	No association	
	1457	986	Hong Kong	.0035	1.19 (1.06–1.33)
	865	1106	West Africa	No association	
Wu et al. [212]	1302	1908	Han Chinese	.033	1.12 (1.01–1.25)
WTCCC [213]	1924	2938	United Kingdom	.020	1.12 (1.02–1.23)
Xiang et al. [214]	521	721	Han Chinese	.016	1.22 (1.04–1.43)
Zeggini et al. [215]	3757	5346	United Kingdom	.0012	1.12 (1.04–1.19)
Meta-analysis Zeggini et al./WTCCC [215]	5681	8284	United Kingdom	.00007	1.12 (1.05–1.18)
Meta-analysis FUSION/DGI/WTCCC/ UKT2D/Zeggini et al. [215]	14,586	17,968	Finland/Sweden/ Poland/USA/UK	.000000053	1.12 (1.07–1.16)

[a] Odds ratio for the minor allele.
[b] European ancestry.

mechanism by which type 2 diabetes is influenced remains to be identified.

There have been several attempts to clarify the role of the polymorphism in SLC30A8 in the development of type 2 diabetes and the focus has been set on insulin secretion due to the importance of ZnT-8 for insulin storage in the granula of pancreatic β cells.

The results are controversial, but there appears to be an association between the risk variant of rs13266634 and reduced insulin secretion. Interestingly, decreased insulin secretion could not be seen usually after oral glucose tolerance test (OGTT), but only after intravenous glucose tolerance test (IVGTT) in European and Chinese individuals [208,214,217–220]. Only Steinthorsdottir et al. [211] observed a reduced corrected insulin response after OGTT, which did not reach significance for females. This corrected insulin response is based on the glucose level and it is the best parameter to evaluate insulin response after OGTT [221]. Furthermore, of the 3982 subjects examined, 231 were type 2 diabetic patients, a fact that could have strengthened significance, since insulin secretion after OGTT might be impaired in diabetic patients due to other mechanisms than those conferred by the risk allele of the polymorphism in SLC30A8. Hyperglycemia, for example, can impair insulin secretion and insulin sensitivity [221,222].

This discrepancy between OGTT and IVGTT concerning the association of SCL30A8 with insulin secretion has not been explained until now, but in some individuals with normal glucose tolerance, the OGTT may not be a sufficient stress to detect subtle defects in insulin secretion [221]. Furthermore, this discrepancy between OGTT and IVGTT is consistent with the finding of Chimienti et al. [52], who showed that enhanced glucose-stimulated insulin secretion compared to control cells could be seen in ZnT-8 overexpressing INS cells only after a high glucose challenge. This suggests that ZnT-8 might be important for insulin secretion triggered by high-glucose stimuli, possibly explaining the association of rs13266634 after IVGTT, which results in higher plasma glucose and may therefore lead to an effect of ZnT-8 on insulin secretion, so that a difference between the risk genotype of SCL30A8 and the minor allele can be detected.

Another important aspect is that the insulin response to a glucose stimulus is biphasic, comprising an early phase of insulin release within the first 10 min followed by a phase of insulin secretion that slowly increases and reaches a plateau after 2–3 h [223]. In patients with type 2 diabetes, the early phase of insulin secretion is often reduced or lost [223] and the biphasic character of insulin secretion might not be easily detected by OGTT, because of the rather gradual rise of plasma glucose due to enteral resorption of glucose compared to intravenous administration. Moreover, the ratio of insulin to glucose measured 15 min after the oral glucose load during OGTT seems to be a better indicator of the first phase of insulin secretion than the insulin/glucose ratio measured after 30 min, but 15 min is usually not part of the sampling protocol of an OGTT [224].

Taking these aspects into consideration, it might be possible that the risk variant in SLC30A8 predisposes to type 2 diabetes by reducing or aborting the first phase of insulin response and/or the insulin response to high-glucose stimuli, which can be detected by IVGTT, but hardly by OGTT.

One study could not show an association between the risk allele in SLC30A8 and insulin secretion during a hyperglycemic clamp at 10 mmol/L glucose in Europeans, although this technique is comparable to an IVGTT except that the plasma glucose concentration is maintained at a constant level during a hyperglycemic clamp, but the sample size was relatively small [225].

Recently, Cauchi et al. [226] found a decreased basal insulin secretion in European individuals carrying the risk allele of SLC30A8. The sample size of this study — 4283 normoglycemic participants were examined — is rather large compared to the studies mentioned above. This fact might have contributed to the detection of a difference in basal insulin secretion, whereas the other studies could only show differences in insulin secretion after a glucose stimulus [226].

In Hispanic Americans and African Americans, the risk variant of SLC30A8 was nominally associated with decreased disposition index, which indicates β-cell function [227].

Kirchhoff et al. [220] examined the conversion of proinsulin to insulin and they were able to demonstrate a significant association between the risk variant of SLC30A8 and reduced proinsulin–insulin conversion, which leads to additionally reduced availability of insulin.

Although genome-wide association studies predominantly reveal an association between the risk allele of SLC30A8 and type 2 diabetes mellitus, ZnT-8 was also shown to play a role in type 1 diabetes mellitus. However, no study genotyping rs13266634 in SLC30A8 in type 1 diabetic patients could show an association between this SNP and type 1 diabetes in contrast to the strong association detected in type 2 diabetes [228–230]. Nevertheless, Gohlke et al. [229] demonstrated that rs13266634 was associated with early onset of type 1 diabetes, indicating that ZnT-8 might not be entirely dispensable in the development of type 1 diabetes.

Indeed, anti-ZnT-8 was identified as a major autoantibody in type 1 diabetic patients and these autoantibodies were present in 60–80% of new-onset type 1 diabetic patients, compared to 2–3% in controls and type 2 diabetic patients [54]. Anti-ZnT-8 as autoantibody is comparable to the already identified autoantibodies characteristic for type 1 diabetes like antibodies to insulin (IAA), the 65-kDa form of glutamate decarboxylase (GADA), protein tyrosine phosphatase IA2 (IA2A) and islet cytoplasmic autoantibodies (ICA) [54,231]. Anti-ZnT-8 is present in 26% of patients who were previously classified as autoantibody negative after being tested for the four autoantibodies enumerated

above. The value of anti-ZnT-8 can be seen in the existence of a fourth measurement additionally to GADA, IA2A and IAA, so that individuals who were classified as individuals at low risk without testing for ZnT-8 would be identified as individuals at risk of developing type 1 diabetes [54].

Since the protein structure that is bound by the autoantibodies lies in the cytoplasm, and since the ZnT-8 autoantibodies emerge relatively late, it is assumed that these antibodies appear secondary to immune damage and are not a primary cause of β-cell destruction [54]. Interestingly, the nonsynonymous SNP rs13266634 is located within the region of highest antibody binding. Hence, Wenzlau et al. [232] examined the relationship between this SNP and ZnT-8 as an autoantigen and they detected that sera from new-onset type 1 diabetic Caucasians reacted with Arg and with Trp and that the restriction to either amino acid corresponded with the respective C or T alleles. Additionally, a strong gene–dosage effect could be observed as both Arg- and Trp-restricted ZnT8 autoantibodies were less prevalent in heterozygotes than in homozygotes. These results argue against molecular mimicry as a basis for β-cell autoimmunity [232].

All in all, these studies suggest that the zinc exporter ZnT-8 and alterations in the coding region of its gene play an important role in the development of type 2 diabetes, as well as in type 1 diabetes, although in the case of type 1 diabetes it might not be involved in the development of diabetes, but in diabetes progression and severity.

6. Conclusion

Considering the beneficial effects of zinc supplementation on glycemic control in type 1 and type 2 diabetic animals and humans, its insulinomimetic effects and the zinc-mediated protection of β cells from damage by immune cells and cytokines, zinc might be regarded as a possible new candidate molecule for diabetes prevention and therapy, especially for type 2 diabetic patients. However, the zinc dose administered has to be determined individually and the zinc status should be controlled in order to prevent adverse effects based on inappropriately high zinc dosage [69,112].

The association of the SNP in SLC30A8, the protein ZnT-8 and the SNPs in the genes of MT with the development of type 2 diabetes, with β-cell function and with the development of diabetic complications offers a potential new way to identify individuals at risk of developing type 2 diabetes, which would allow preventive measures, and anti-ZnT-8 can be seen as a new diagnostic tool for the diagnosis of type 1 diabetes.

In conclusion, diabetes mellitus can be regarded as a multifactorial disease with various contributing factors, but nevertheless, zinc status consistently seems to be adversely affected regardless of the causal mechanisms leading to diabetes. The involvement of zinc in multiple processes of the development and progression of diabetes mellitus emphasizes the diabetogenic effect of a disturbed zinc homeostasis and provides new possibilities of diagnosis, prevention and therapy.

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