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Zinc, pancreatic islet cell function and diabetes: new insights into an old story

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Abstract

Zn is an essential trace element, involved in many different cellular processes. A relationship between Zn, pancreatic function and diabetes was suggested almost 70 years ago. To emphasise the importance of Zn in biology, the history of Zn research in the field of diabetes along with a general description of Zn transporter families will be reviewed. The paper will then focus on the effects of Zn on pancreatic β-cell function, including insulin synthesis and secretion, Zn signalling in the pancreatic islet, the redox functions of Zn and its target genes. The recent association of two 'Zn genes', i.e. metallothionein (*MT*) and Zn transporter 8 (*SLC 30A8*), with type 2 diabetes at the genetic level and with insulin secretion in clinical studies offers a potential new way to identify new drug targets to modulate Zn homeostasis directly in β-cells. The action of Zn for insulin action in its target organs, as Zn signalling in other pancreatic islet cells, will be addressed. Therapeutic Zn–insulin preparations and the influence of Zn and Zn transporters in type 1 diabetes will also be discussed. An extensive review of the literature on the clinical studies using Zn supplementation in the prevention and treatment of both types of diabetes, including complications of the disease, will evaluate the overall beneficial effects of Zn supplementation on blood glucose control, suggesting that Zn might be a candidate ion for diabetes prevention and therapy. Clearly, the story of the links between Zn, pancreatic islet cells and diabetes is only now unfolding, and we are presently only at the first chapter.

Key words: Zinc: Panceatic islets: Diabetes: Zinc transporters

Insulin, diabetes and zinc research

Diabetes is one of the most prevalent chronic diseases, the hallmark of which is hyperglycaemia due to a lack of insulin secretion and/or action. Insulin, the glucose-lowering hormone secreted by pancreatic β-cells, was discovered in 1921 in Toronto by Best and Banting, after they isolated pancreatic extracts without contamination of the tissue extract with digestive enzymes[1]. These extracts, when injected in a pancreatectomised, diabetic dog, caused a huge drop in blood glucose levels. The substance isolated from pancreatic islets, first called isletin, became rapidly known as insulin. Soon after this discovery, the insulin preparation was successfully tested on a 14-year-old diabetic patient at the Toronto General Hospital. Best and colleagues continued improving the pancreatic extract and eventually managed to produce enough for the hospital's demand. Crystalline insulin was isolated in 1926, using a highly buffered solution containing several substances as the crystallising medium[2]. The very nature of the substance(s) promoting crystallisation and the mechanisms of crystal formation remained unclear despite it being known that the pancreas contains high amounts of Zn. A few years later, Scott discovered that adding Zn to a phosphate-buffered solution containing insulin induced the formation of characteristic rhombohedral insulin crystals[3]. He then showed a direct effect of Zn ions on the action of insulin[4]. Because of the close association between insulin and Zn, Scott's next idea was to estimate the Zn content in the pancreas of a series of normal and diabetic individuals[5]. Interestingly, he found that the amount of Zn contained in the pancreas of diabetics is only one-half that of healthy subjects, while there was no difference in the liver Zn concentration, raising the possibility that at least part of the Zn in the pancreas could be concerned with the storage of insulin. Thus was born our understanding of the link between insulin and Zn.

A second breakthrough in the diabetes field came from the discovery of the structure of insulin. Following Scott's discovery that the insulin preparations from different species had the same effect, it has been suggested that different insulins behave as a single molecule in solubility studies, despite some differences in some amino acids[6]. In 1955, Sanger and colleagues determined that conserved

Abbreviations: HbA1c, glycated Hb; MT, metallothionein; SLC30A, solute carrier 30A; ZIP, Zrt-like, Irt-like protein; ZnT, zinc transporter.

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amino acids and important disulfide bridges might be implicated in insulin activity, since insulin was inactivated by any treatment affecting those sulfur bonds[7]. After many years of research that brought new insights to our understanding of the structure of insulin, including the determination of single-chain amino acid composition and X-ray photographs of single insulin crystals, Adams *et al.*[8] eventually determined the crystal structure at a resolution of 2.8 Ångström. They showed that the crystal was formed by six insulin molecules and two Zn atoms and determined the intramolecular Zn coordination spheres.

Nevertheless, the physico-chemical interactions between Zn and insulin had been known for decades before the crystal structure of Zn–insulin (2:6) was resolved. As early as in the 1930s, it was clear that the addition of Zn to insulin delayed its action when injected into diabetic patients. Indeed, Zn ions were rapidly added to insulin *in vitro* to produce protamine Zn insulin (PZI) and used in clinics[9]. However, PZI is now rarely used in human patients, but is instead used by veterinarians, especially to treat cats with diabetes. After the addition of Zn ions to insulin preparations, the quantity of insulin necessary to control blood glucose was found to be significantly reduced, thus requiring fewer injections[10]. Therefore, laboratories rapidly aimed to develop different insulin preparations that have a faster onset to complement the longer-lasting action. NPH insulin (or neutral protamine Hagedorn), which is still on the market, has the advantage of being possibly mixed with a fast-acting insulin to complement its longer-lasting action[11]. Removing Zn to avoid crystallisation and accelerate the onset of insulin action is definitely a way to formulate fast-acting insulin preparations. Indeed, it has been shown recently that insulin glulisine (3^{B}-Lys, 29^{B}-Glu-human insulin) has the most rapid onset of action because of its Zn-free formulation[12]. Regular plus NPH insulins are the preferred mixture of rapid- and intermediate-acting insulins because the effect of the combined insulins is the same as that of regular and NPH insulin injected separately[13].

Despite Zn being recognised as an important ion for insulin crystallisation and action, the molecular mechanisms of its mode of action remained poorly understood. Furthermore, research was limited by the lack of tools available to investigate Zn homeostasis in the β-cell by physiologists. Moreover, in the mid-1960s, the second messenger Ca was shown to potentiate insulin secretion[14]. Researchers soon established the now well-known Ca dependency of glucose-induced insulin release[15], and Ca probes were developed, which boosted research on insulin secretion by the β-cell[16]. Indeed, from the early 1970s, Ca has been revealed as one of the most important protagonists in the field of diabetes and insulin secretion; Zn became less important for diabetologists. Intracellular Ca^{2+} concentration and fluctuations (oscillations) are known to regulate key cellular and signal-transduction processes; in regard to β-cell signalling, Ca is the key ion for both triggering and amplifying insulin secretion (for a review, see Henquin[17]).

In the mid-1990s, the development of Zn-specific fluorescent probes, concomitantly, though independently to the identification of Zn transporters, represented a leap forward in Zn research. Emphasising that studies on intracellular Ca, as other important ions, have been greatly facilitated by the use of fluorophores, Zalewski *et al.*[18] synthesised a membrane-permeant fluorophore specific to Zn^{2+}, Zinquin. In 1994, they used this intracellular Zn probe, which allows real-time observation of exchangeable Zn in live cells, to reveal labile Zn in pancreatic islet cells[19]. Importantly, they showed that the Zinquin signal responded to stimulation of islet cells with a high concentration of glucose, i.e. inducing insulin secretion decreased the islet cells' content of labile Zn. Probes similar to those of Ca were now available for use by the research community. Concomitantly, the very first mammalian Zn transporter was cloned and characterised[20]. In this seminal paper, Palmiter & Findley[20] established in 1995 the main characteristics of the Zn transporters ZnT (SLC30A; solute carrier 30A), by describing a six-transmembrane domains protein, with a large intracellular loop and a C-terminal tail, which was suggested to function as a multimer and transport Zn from the cytosol to the extracellular space. The same group, 1 year later, paved the way to the identification of the ZnT protein family of Zn transporters, by identifying two other proteins, named ZnT2 and ZnT3[21,22]. In 1998, the group led by Eide reported the cloning of the first Zn transporter genes from the ZIP (Zrt-like, Irt-like protein; SLC39A, solute-carrier-39A) protein family of Zn transporters, namely the *ZIP1*, *ZIP2* and *ZIP3* genes of *Arabidopsis thaliana*[23]. Transporting Zn in the opposite direction of ZnT, ZIP transporters carry Zn ions across cellular membranes from the extracellular space – or intracellular organelles – to the cytosol. This study led to the identification of a family of up to fourteen related proteins (for a review, see Eide[24]). With the cloning of an increasing number of Zn transporters and development of fluorescent probes[25], a new era opened for Zn research. Indeed, the last 20 years has witnessed overall very rapid progress in our understanding of intracellular Zn homeostasis, therefore contrasting with the slow tempo observed during the 1950s–1980s.

It is now well established that cells control uptake and excretion of Zn^{2+} through two different families of proteins: the *SLC39A* and *SLC30A* genes, which encode for ZIP and ZnT, respectively[26]. To date, up to twenty-four Zn transporters have been described, most of which have relevance to clinical science. Some transporters have ubiquitous expression, while others are restricted to a few tissues, which led to the question of why there are so many Zn transporters, compared with those needed for other ions such as Cu or Fe[27]. Such a large panel of ZnT and ZIP transporters both serves a housekeeping role in cellular Zn homeostasis and participates in cell signalling. Indeed, Zn transporters play important physiological

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roles, for example, during embryogenesis, cell division and migration, and have a specific role in different organ systems, including but not restricted to the brain, immune system, skin and pancreas (for a review, see Plum *et al.*[28]). The Zn transporter ZIP4, expressed at the apical surface of intestinal enterocytes and visceral endoderm cells, responds to Zn levels and translocates from cytoplasmic vesicles to the plasma membrane to enhance Zn uptake during Zn deficiency, suggesting that Zn-regulated intracellular trafficking of Zn transporters is an important mechanism for the control of dietary Zn absorption, and cellular Zn homeostasis[29]. Other members of the ZIP family have been shown to be activated post-translationally by phosphorylation, and are strongly implicated in cell signalling. In response to extracellular Zn or epidermal-growth-factor/ionomycine treatment, the endoplasmic reticulum Zn transporter ZIP7 is phosphorylated on conserved residues by protein kinase CK2, leading to the release of intracellular Zn stores and subsequent activation of protein kinase B (Akt), and extracellular signal-regulated kinases 1 and 2 (ERK1/2)[30]. ZIP7 is therefore a key protein for Zn signalling during proliferative responses and cell migration. A member of the ZnT family, ZnT1, levels of which rapidly increase after global ischaemic injury, is associated with long-life (L)-type Ca channels, thus leading to downstream activation of ERK and heart protection after ischaemia–reperfusion injury[31]. Indeed, at the level of the organism, Zn transporters play a crucial role in maintaining adequate Zn homeostasis in all organs. Some mutations that affect particular transporters can lead to genetic disorders, or susceptibility to diseases. Mutations in *ZIP4* are responsible for acrodermatitis enteropathica (Online Mendelian Inheritance in Man OMIM 201100; http://www.omim.org/entry/201100), a rare autosomal recessive disease in which patients suffer from a severe general Zn deficiency resulting from defective uptake of Zn in the intestine[32]. In this case, a lifelong treatment in the form of Zn supplementation, typically 1–3 mg Zn/kg administered orally per d, is sufficient to eliminate the symptoms[33]. Proteins controlling the cellular availability of Zn are also involved in diabetes, for which susceptibility loci have been identified in the genes encoding for ZnT8 and metallothionein (MT) 1A[34,35] (see below). However, an exhaustive and comprehensive description of Zn homeostasis-regulating proteins is out of the scope of the present review. Hence, the paper will focus preferentially on the Zn transporters that have been shown to be crucial for islet cell function; for specific reviews on Zn transporters, see Cousins & Lichten[27] and Kambe[36].

Zinc and the pancreatic β-cell

Insulin is synthesised and stored in the pancreatic β-cell in a solid form, as a Zn–insulin (2:6) crystal[37]. Hence, the pancreatic β-cell is one of the cell types that contain the highest quantities of Zn. It is estimated that the Zn content within insulin granules is in the millimolar range, for example, 10–20 mM[38]. As an enzymic cofactor, Zn^{2+} is also implicated in all processes of synthesis, storage and secretion of insulin, as well as being a signalling molecule after insulin secretion[39].

After synthesis in the reticulum, pro-insulin is transported to the Golgi where immature, secretory 'progranules' are formed. Both pro-insulin and insulin associate with Zn, and it has been shown that the formation of pro-insulin–Zn hexamers is fundamental for its processing to insoluble insulin–Zn crystals[40]. Therefore, a sufficient amount of Zn in the β-cell, particularly in insulin granules, is required for the correct hexamerisation and processing of insulin. Pancreatic β-cells express most of the Zn transporter proteins[41], which ensure basal Zn homeostasis required for providing Zn to all Zn proteins, for example, Zn enzymes and transcription factors. Among them, ZnT5 is a Zn transporter more abundant in pancreatic β-cells than in other tissues. It is expressed in endoplasmic reticulum and the Golgi apparatus, and thus may have an important function in the β-cell[36,42]. Another important Zn transporter in the β-cell might be ZnT3, which is highly expressed in the brain but is also present in different organs such as the testis, retina, prostate and pancreas (for a review, see Smidt & Rungby[43]). ZnT3 was shown to be up-regulated by glucose in a concentration-dependent manner, and glucose metabolism is affected *in vivo* in ZnT3 knock-out mice[44]. The same group showed more recently that silencing of ZnT3 in INS-1E cells significantly increased cell death, while both insulin content and secretion were decreased[45].

Contrasting with the ubiquitous expression of some other ZnT, the Zn transporter ZnT8 has a unique expression profile, being almost exclusively restricted to pancreatic islets[46]. However, it has been reported to be expressed, though at very much lower levels, in other endocrine cells such as adipocytes[47], epithelial cells within thyroid follicles and in the adrenal cortex[48]. Its unique expression profile suggests a primary physiological role to the pancreatic islet cells, for which it is a major component for both Zn accumulation in the insulin granules (Fig. 1) and regulation of insulin secretion[49]. Interestingly, *SLC 30A8*, the gene encoding for ZnT8, has been implicated in the development of type 2 diabetes in man by recent genome-wide association studies[35,50]. This polymorphism, for which the at-risk allele corresponds to the polymorphic variant rs13266634, is associated with a 53% increased risk of developing diabetes. The transition T-C in the coding region of *SLC30A8* induces in the protein a change in amino acid, from a tryptophan to arginine at position 325, which impairs the Zn transport activity of the protein[51]. The link between impaired β-cell function and decreased Zn transport activity by ZnT8 has also been reported *in vitro* and in clinical studies[45,52]. For example, ZnT8 has been shown to affect insulin secretion in a Danish population, where homozygous carriers of the risk allele had an estimated 22% lower insulin response

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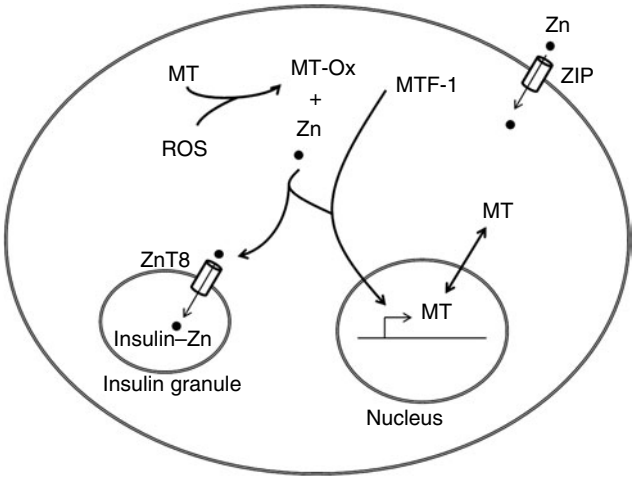

Fig. 1. Mechanisms of zinc homeostasis in the β-cell: intracellular free cytosolic zinc, which can be either imported through Zrt-like, Irt-like protein (ZIP) transporters or released from metallothionein (MT) during oxidative stress, can be imported into insulin granules through zinc transporter ZnT8 to form crystalline insulin–zinc hexamers. Zinc can also bind to metal-responsive transcription factor-1 (MTF-1), which translocates to the nucleus and further up-regulates MT synthesis. MT-Ox, oxidised MT; ROS, reactive oxygen species.

than carriers of the protective allele[53]. Similarly, Staiger *et al.* demonstrated that rs13266634 is associated with reduced insulin secretion stimulated by intravenously administered glucose[54], suggesting that rs13266634 in *SLC30A8* is a crucial allele for β-cell function. Moreover, the R325W non-synonymous polymorphism in ZnT8 has been shown to protect against post-transplantation diabetes mellitus[55], a major metabolic complication in renal transplant recipients, for which insulin-secretory defects play an important role in pathogenesis. The same polymorphism in *SLC30A8*, rs13266634, has eventually been associated with glycated Hb (HbA1c), a marker of long-term blood glucose levels, in a non-diabetic population[56]. Altogether, an increasing number of studies have confirmed that the SNP rs13266634 is among the most confirmed genetic markers of type 2 diabetes in Europeans and East Asians[57].

Mice displaying global or β-cell-specific deletion of ZnT8 have been studied[51,58–60]. Despite slightly different metabolic phenotypes between laboratories, these studies reported a massive reduction in the capacity of pancreatic islets to store Zn, a lack of crystalline insulin in β-cells, impaired glucose-induced insulin secretion and glucose tolerance abnormalities. It is noteworthy that silencing ZnT8 expression in the INS-1 β-cells led to similar results, i.e. reduced insulin content and glucose-inducible insulin secretion[61]. Moreover, the effects of high-fat diet feeding on ZnT8-null mice showed that global loss of ZnT8 is involved in exacerbating diet-induced obesity and resulting insulin resistance[62]. Since ZnT8 is also expressed in other tissues and in pancreatic α-cells, the effects of ZnT8 on obesity and insulin resistance might be due to non-β-cell-specific effects. Overall, these studies support the increasing body of literature that suggests that ZnT8 is crucial for insulin processing and secretion.

To highlight the importance of correct Zn homeostasis for pancreatic islets, another Zn-binding protein, MT, has resulted in numerous research studies describing the effects of Zn on reducing diabetic complications associated with oxidative stress[63]. MT are intracellular low-molecular-weight, cysteine-rich proteins with potent metal-binding capacity (for a review, see Maret[64]). MT can buffer and distribute Zn to apoproteins, including transcription factors, since they can shuttle from the cytosol to cellular compartments such as the nucleus[65]. MT also have redox functions, which are made possible by their thiolate coordination environments. It has been shown that overexpression of MT in transgenic mice could protect from streptozotocin-induced diabetes, mainly because of their scavenging properties against reactive oxygen species[66]. More recently, Park *et al.*[67] showed that intraperitoneal injection of the Tat-MT protein delayed the development of diabetes in streptozotocin-treated mice and improved insulin secretion in rats by decreasing the formation of reactive oxygen species and subsequent DNA fragmentation[67]. Indeed, MT provides a mechanism whereby cellular Zn buffering and availability and redox metabolism are linked[68]. When oxidants react with thiolate clusters of MT, Zn ions are released and the oxidised protein is formed (Fig. 1). Moreover, released Zn can up-regulate MT synthesis through nuclear translocation of the metal-responsive transcription factor-1, which acts as a sensor to up-regulate Zn-binding proteins[69]. Polymorphisms in *MT1A* have been linked to the susceptibility of diabetes and cardiovascular complications. In a recent study, the +647 A/C *MT1A* polymorphism was linked to a modulation of MT levels, to an increase in Zn release and to type 2 diabetes[70]. Interestingly, previous work from the same group already linked a SNP in the promoter region of the *MT2A* gene (209 A/G *MT2A*) with hyperglycaemia and increased HbA1c[71]. Importantly, the work of the Mocchegiani group[72], by studying polymorphisms affecting Zn release by MT, provided evidence for their involvement in some pathogenic mechanism of type 2 diabetes and its complications, essentially cardiovascular outcomes[72].

Consequently, proteins implicated in Zn storage within β-cells, such as ZnT8 or MT, are crucial to protect the β-cell mass from cell death during diabetes. MT, along with the high content of Zn in the pancreatic β-cells therefore represent a mechanism by which β-cells are protected from cell death. Indeed, Zn depletion, a condition often observed during diabetes, may induce apoptosis by itself[73] and/or promote oxidative stress-induced apoptosis[74]. However, Zn could act as a double-edged sword, and Zn overload can also induce apoptosis in pancreatic β-cells[75]. In this case, Zn homeostasis proteins such as ZnT8 or MT could also protect cells by sequestering Zn either in intracellular vesicles or through thiolate clusters.

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Hence, the strict preservation of 'free' Zn levels in the β-cells is crucial in the context of glucose regulation of Zn concentrations. Indeed, a decrease in intracellular free Zn in islet cells in response to high concentration of glucose has been observed with the Zn-specific fluorescent probe Zinquin[19]. However, a more recent study reported an increase in cytosolic free Zn concentration within primary β-cells[76]. Such a discrepancy in the changes of Zn concentration by glucose can be explained by either the nature and localisation of the probes used for these studies, the nature of the model, i.e. isolated β-cells or pancreatic islets, and/or other factors. Interestingly, the latter study hypothesised that the increase in cytosolic free Zn concentrations may be due to the uptake (or reuptake) of Zn, and/or to Zn release by MT upon oxidative stress induced by high glucose. Indeed, maintaining adequate intracellular and intragranular Zn levels facilitates insulin synthesis and processing as well as its storage, for example, crystallisation.

Zinc and type 1 diabetes

Type 1 diabetes is an organ-specific auto-immune disease in which the body's immune system specifically destroys pancreatic β-cells, so that the pancreas is no longer able to produce insulin. Immune attack of the pancreas by specific T cells also results in the production of auto-antibodies, which are both causal to the disease and used for diagnostic purposes. The most common auto-antibodies are directed against insulin, glutamic acid decarboxylase (GAD65) and islet cell antigen-2 (IA-2, a tyrosine phosphatase-like protein), all of which are intracellular proteins relatively specific to pancreatic β-cells. They also share the property to be elements of the insulin secretion pathway[77].

As for type 2 diabetes, a hallmark of type 1 diabetes is hypozincaemia. Taking into account the importance of Zn for the correct functioning of the immune system (for a review, see Haase & Rink[78]), a drop in plasma Zn levels during diabetes may aggravate the disease. However, it is noteworthy that Zn supplementation studies attenuate the disease (see below). Importantly, an innovative work by Hutton and colleagues recently identified a fourth major common auto-antigen: the Zn transporter ZnT8[79]. Inclusion of ZnT8 auto-antibodies (ZnT8Ab) in diagnostic tests was found to increase the diagnostic specificity of type 1 diabetes, raising detection rates to 98% at disease onset[80]. Moreover, a very recent study reported that ZnT8 is also recognised by autoreactive $CD8^+$T cells, which play a central role in diabetes pathogenesis[81]. Interestingly, the main epitopes for auto-antibodies against ZnT8 are defined in the region of amino acid 325, i.e. the same polymorphic amino acid linked to type 2 diabetes (see above). Moreover, genotype analysis in type 1 diabetic patients showed that patients with a diabetes onset before the age of 5 years had an increased prevalence of the cytosine (C) allele (the at-risk allele for type 2 diabetes) compared with patients who developed type 1 diabetes after the age of 5 years[82], suggesting that genetic susceptibility for β-cell dysfunction in the presence of autoimmunity may lead to early manifestation and accelerated progression of the disease.

Some insulin-dependent type 1 diabetic patients are eventually grafted with human islets to avoid insulin dependency. However, the success of transplantation depends largely on the survival of the transplanted islets. Using an innovative method, Kerr-Conte *et al.*[83] improved the quality of pre-transplant human islets and increased islets viability by adding zinc sulfate as a supplement in the culture medium. Remarkably, in an original study using diabetic rats as recipients for syngeneic islets, Okamoto *et al.*[84] showed that a Zn-rich environment significantly improved transplanted islet survival. Indeed, they showed that in rats supplemented with Zn, not only were plasma Zn levels higher than in controls, but early graft loss was decreased and blood glucose levels were lower than in controls, suggesting that a Zn-rich environment is advantageous for the recipient during intraportal islet transplantation.

The effect of zinc in insulin target organs

The insulinomimetic effect of Zn has been known for decades. Indeed, as early as in 1980, Zn was shown to exert a potent stimulatory effect upon lipogenesis *in vitro*[85]. Zn-stimulated lipogenesis by adipocytes was found to be independent of and additive to that of insulin. Further studies indicated that Zn ions stimulate glucose transport and glucose oxidation[86]. Interestingly, Zn increases both lipogenesis and glucose transport through activation of the entire insulin-signalling pathway, including activation of mitogen-activated protein kinases and protein kinase B (Akt), a serine/threonine-specific protein kinase that is crucial for glucose metabolism. This insulinomimetic effect of Zn has been implicated in the glucose-lowering effect of synthetic insulins (see above); insulinomimetic Zn complexes have been synthesised and evaluated both *in vitro* and *in vivo* in diabetic animal models[87]. Moreover, oral administration of Zn complexes that increase Zn absorption from the gastrointestinal tract have been found to significantly improve hyperglycaemia, glucose intolerance and insulin resistance in KKA^y mice, an obesity-linked type 2 diabetic mouse model, strongly suggesting that increased Zn absorption, or Zn supplementation therapy, is helpful in decreasing blood glucose levels in diabetes[88]. Activation of insulin signalling through phosphorylation of Akt by Zn insulinomimetics occurs in a concentration- and time-dependent manner. The Zn-dependent effect of small molecules on insulin signalling has been shown to act on downstream effectors such as the transcription factor FOXO1a (forkhead box protein O1a) and the key gluconeogenic regulatory

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enzymes phosphoenolpyruvate carboxykinase and glucose 6-phosphatase[89]. Zn insulinomimetics eventually induce the translocation of the GLUT4 protein to the plasma membrane, where it promotes glucose transport from blood to muscle cells and adipocytes[90].

Since Zn activates the whole pathway of insulin signalling, it has to act very early on in this pathway, at or close to the insulin receptor. Indeed, the phosphorylation of three tyrosine residues central to the activity of the insulin receptor is increased by Zn treatment[91]. Conversely, Zn chelation reduces phosphorylation of the insulin receptor upon insulin treatment of C6 rat glioma cells. Experiments using Zn ionophores excluded the interaction of Zn with the extracellular domain of the insulin receptor, suggesting that the effect of Zn in the phosphorylation of the insulin receptor occurred intracellularly. Hence, Zn has been shown to inhibit protein tyrosine phosphatases (PTP). PTP 1B, the key phosphatase implicated in the dephosphorylation of the insulin receptor, has a half-maximal inhibitory concentration (IC_{50}) in the range of physiologically available Zn levels, i.e. in the low nanomolar range[92]. Moreover, kinetic analysis revealed that Zn ions are reversible inhibitors of the cytoplasmic catalytic domain of the receptor protein-tyrosine phosphatase β[93]. Here, again, inhibition is in the range of intracellular free Zn ion concentrations. Hence, intracellular, available free Zn levels regulate insulin signalling; this may be a crosstalk provided by Zn ions between the cell's response to glucose levels and redox state of the cell. Indeed, glucose metabolism induces the production of reactive oxygen species, especially H_2O_2, which in turn can oxidise MT and increase free Zn levels (see above), thereby providing a means to fine tune insulin signalling and glucose transport into the target organ cells. Since pancreatic β-cells also express the insulin receptor, the Zn co-secreted with insulin, and re-uptaken mainly by long-life (L)-type Ca channels[94], may act as an autocrine signalling ion for β-cell glucose metabolism.

Zinc signalling and α-cells

Pancreatic α-cells secrete glucagon, a hormone that has the opposite action to that of insulin. Glucagon is released during hypoglycaemia, and increases blood glucose levels by stimulating hepatic glucose output[95]. Among different mediators of α-cell function, Zn, as insulin, has been proposed to be a paracrine signalling molecule co-secreted with insulin from β-cells despite some contradictory results in different studies[96]. Since Zn can exert a strong modulatory effect on synaptic function in the brain (for a review, see Sensi *et al.*[97]), the hypothesis that Zn might regulate glucagon secretion was first tested in isolated pancreas[39]. Zn was found to have an inhibitory action on glucagon secretion. Studies from the same group revealed that in isolated α-cells, the mechanism by which exogenous Zn inhibited glucagon secretion resulted from direct activation of K (K_{ATP}) channels[98]. However, monitoring of free cytosolic concentrations of ATP and Ca both in α-cells and intact islets confirmed the effect of insulin but failed to reveal any effect of Zn on the suppression of glucagon secretion by glucose[99]. Using the perifusion technique and drugs that manipulates K_{ATP} channels, Robertson *et al.*[100] confirmed that Zn interacts at K_{ATP} channels to provide tonic suppression of glucagon secretion. Nevertheless, when studying glucagon secretion in islets isolated from β-cell-specific ZnT8 knockout mice, which contain and secrete less Zn than wild-type islets, no effect on glucagon secretion was observed, though the ability of exogenous Zn to inhibit glucagon secretion was still preserved in these islets[96]. This discrepancy in some experiments suggested that an overlap or redundancy in the mechanisms of inhibition of glucagon secretion might exist. Further studies will be clearly needed to fully understand the interaction between Zn and the other paracrine factors and/or direct effects of glucose on α-cells.

Dietary zinc supplementation and diabetes

The predominant effect of diabetes on body Zn homeostasis is to induce hypozincaemia, hyperzincuria, decreased gastrointestinal absorption of Zn or even increased urinary excretion[101]. As early as in the 1930s, Scott discovered that the amount of Zn contained in the pancreas of diabetics patients was only one-half that of non-diabetics[5]. In a diabetic population, serum Zn levels were significantly reduced as compared with controls[102]. In patients with type 1 diabetes, Zn concentrations in erythrocytes presented lower than normal values[103]. Moreover, a significant reduction of serum Zn in both type 1 and type 2 diabetic patients was very recently confirmed by the group of Rink. In this case, Zn supplementation elevated intracellular Zn concentrations and increased insulin signalling[104].

Since Zn plays a crucial role in the processes of synthesis, storage and secretion of insulin (see above), the hypozincaemia observed in diabetes might worsen the diabetic condition, especially for type 2 diabetes. Moreover, Zn deficiency will increase intracellular oxidants and free radical production, while concomitantly decreasing Zn-dependent antioxidant enzymes and MT expression, thereby affecting the viability of the islet cells and impairing the situation a little further[105]. Therefore, it has been suggested that oral Zn supplementation may have a role in therapy, since an overall analysis of the scientific literature advocates beneficial effects on both glycaemic control and lipid parameters[106]. Reduced pancreatic Zn content is also evident in several genetic mouse models of type 2 diabetes; Zn supplementation has led to promising results. In mice carrying a mutation in the leptin receptor (*db/db* mice), Zn supplementation has been shown to normalise pancreatic Zn levels and attenuate hyperglycaemia and hyperinsulinaemia[107]. Similarly, Zn supplementation in

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ob/ob mice (mutation in the leptin gene) increased islet insulin content and attenuated fasting hyperglycaemia[108].

However, Zn supplementation in human subjects has yielded contradictory results, principally because the dosage and the Zn species used were different. In the very first study of Zn supplementation in diabetic individuals, no correlation between serum Zn and HbA1c levels was found. Zn supplementation for 8 weeks did not affect HbA1c levels in patients, though twenty times the daily recommended dose (usually 10 mg/d) was administered[109]. Importantly, in a study confirming that hypozincaemia was mainly due to increased zincuria in diabetics, dietary Zn was even found to aggravate glucose intolerance[110]. A few years later, another study reported a statistically significant increase in insulin and serum Zn levels, along with a concomitant decrease in fasting blood glucose after 3 weeks of dietary Zn supplementation in diabetic patients[111], suggesting that supplementation with Zn might be useful to reduce plasma glucose in diabetics. However, even though the beneficial effect of Zn supplementation for blood glucose and HbA1c levels has been confirmed, the effect of Zn supplementation on insulin levels still remains controversial[112]. A study conducted in India on type 2 diabetes mellitus patients with neuropathy also found that supplemental zinc sulfate given orally for 6 weeks normalised Zn and blood sugar levels[113]. However, in this case the very high dosage for the study, i.e. 600 mg/d, limits extrapolation to dietary supplementation and comparison with other reports. Indeed, in another study on type 2 diabetes patients suffering neuropathy supplemented with comparable dose and duration (660 mg zinc sulfate/d; 6 weeks) a better glycaemic control was observed, along with an improvement in peripheral neuropathy[114]. Approximately the same dosage (200 mg three times per d) for 2 months was used by Marchesini *et al.*[115], who could thus show in patients with cirrhosis that long-term oral Zn supplementation normalised plasma Zn levels and improved glucose tolerance[115].

In additional studies with slightly higher than normal daily requirements, supplemental Zn in diabetics both restored serum Zn levels and significantly decreased the mean value for HbA1c percentage at the end of the 3 months of follow up, while no significant changes were found in the control group[116]. Similarly, treatment of diabetic patients with 50 mg zinc gluconate/d improved both fasting plasma glucose and HbA1c percentage levels[117]. However, in the latter report C-peptide levels were not affected, suggesting that improvement of the diabetic condition takes place by a mechanism different from that of increased insulin secretion, possibly through an increase in insulin sensitivity. In a very recent study, Zn supplementation improved glycaemic control measured by HbA1c percentage and both fasting and postprandial glucose. Furthermore, Zn supplementation also lowered serum cholesterol and cholesterol:HDL ratio, suggesting that Zn may inhibit the activation of oxidative stress-responsive proteins[112]. To further highlight the beneficial effects of Zn supplementation in type 2 diabetes, a pioneering study by Faure *et al.* showed that after 3 months of zinc gluconate treatment (30 mg daily), markers of oxidative stress, including lipid peroxidation markers, were decreased and antioxidant enzyme activity was increased, suggesting a protective effect of Zn for pancreatic β-cells[118]. Two others studies confirmed this antioxidant effect of Zn supplementation in patients with type 2 diabetes mellitus[119,120]. Moreover, during a 5-year follow-up of antioxidant supplementation in type 2 diabetic retinopathy, the retinopathy stage showed a retardation of progression in the subgroup with supplementation, along with preservation of its antioxidant plasma status levels[121]. Diabetes induces oxidative stress through hyperglycaemia and hyperlipidaemia, both of which cause damage to multiple organs, thus leading to various complications. The innovative work of Cai and colleagues on the role of Zn in diabetic complications showed that the ability of Zn to induce MT significantly protects heart and kidney against diabetes-induced pathophysiological changes[122], thereby suggesting that Zn supplementation may play an important role in the prevention of the development of diabetes and its complications.

However, it should be noted that the analysis of dietary and total Zn intake studies might be complicated by the existence of polymorphisms in *SLC30A8* and *MT1A* linked to type 2 diabetes. In fact, a meta-analysis of fourteen cohort studies identified a nominally significant interaction between total Zn intake and a *SLC30A8* variant on fasting glucose levels[123]. This analysis suggested that Zn intake might modify the effects of glucose-raising genetic loci. In other terms, it might be of importance to take into account gene–environment interactions in future studies on total and dietary Zn intake and diabetes.

Conclusion and perspectives

The interest of the scientific community in the role of Zn in diabetes has been continually increasing, from studies investigating the effect of Zn supplementation in the prevention, treatment and complications of diabetes (for example, renal failure, vision disorders, macrovascular complications) to the discovery of polymorphisms in Zn genes, for example, *ZnT8* and *MT*, linked to diabetes by recent genome-wide association studies. In the last decade, genetic and functional studies have allowed a better understanding of the importance of Zn for pancreatic islet cells at the molecular level. It is now obvious that Zn has beneficial effects in many steps of diabetes pathophysiology, including insulin synthesis and secretion, β-cell function and mass, islet cell communication, protection of complications, and immune system modulation in type 1 diabetes. The overall beneficial effects of Zn supplementation on blood glucose control in both types of diabetes suggest that Zn is a candidate ion for diabetes prevention and therapy. Zn supplementation could be a

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simple way to improve clinical parameters, for example, blood glucose and lipid profile, in diabetics; ZnT8 and/or MT might be promising therapeutic targets for the treatment of type 2 diabetes. Nevertheless, more studies are needed to unravel the exact role(s) of Zn ions in the pancreatic β-cell and in islet cell-to-cell communication. Moreover, future clinical studies with Zn supplementation normalized with gene–environment interaction are necessary to evaluate accurately the role of dietary Zn and to compare results of different studies between diverse populations and/or dosage.

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