Zinc transporters and their role in the pancreatic \(\beta\)-cell

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ABSTRACT

Zinc is an essential nutrient with tremendous importance for human health, and zinc deficiency is a severe risk factor for increased mortality and morbidity. As abnormal zinc homeostasis causes diabetes, and because the pancreatic \(\beta\)-cell contains the highest zinc content of any known cell type, it is of interest to know how zinc fluxes are controlled in \(\beta\)-cells. The understanding of zinc homeostasis has been boosted by the discovery of multiple protein families of zinc transporters, and one of them – zinc transporter 8 (ZnT8) – is abundantly and specifically expressed in the pancreatic islets of Langerhans. In this review, we discuss the evidence for a physiological role of ZnT8 in the formation of zinc-insulin crystals, the physical form in which most insulin is stored in secretory granules. In addition, we examine this information, collected in genetically modified mouse strains, to the knowledge that genetic variants of the human ZnT8 gene predispose to the onset of type 2 diabetes and that epitopes on the ZnT8 protein trigger autoimmunity in patients with type 1 diabetes. The overall conclusion is that we are still at the dawn of a complete understanding of how zinc homeostasis operates in normal \(\beta\)-cells and how abnormalities lead to \(\beta\)-cell dysfunction and diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00199.x, 2012)

KEY WORDS: Insulin crystallization, Pancreatic \(\beta\)-cell, Zinc transporter 8

INTRODUCTION

Zinc is an essential element for all cells, as it is used as a cofactor for many enzymes and transcription factors that mediate key cellular processes, such as protection against oxidative stress, deoxyribonucleic acid (DNA) repair, DNA replication, differentiation and apoptosis\(^1\)\(^\tilde{\text{5}}\). Zinc homeostasis is regulated by the coordination between zinc import, export and distribution. The total zinc content per cell differs according to cell type, and mammalian pancreatic \(\beta\)-cells have one of the highest zinc concentrations of the whole organism, with estimated values between 10 and 20 mmol/L cell space in insulin-containing vesicles\(^4\). As the mammalian body has no – or only limited – zinc stores, it depends on daily zinc intake\(^5\)\(^,\)\(^,\)\(^6\). Inadequate zinc intake is an important health problem, as the prevalence is approximately 20.5% of the world’s population\(^7\). As a dramatic result, zinc deficiency is one of the most important diet-related factors in human disease, and estimated to be responsible for 0.4 million child deaths per year\(^8\). Impaired zinc homeostasis is linked to several diseases, including diabetes mellitus\(^9\)\(^,\)\(^10\). Furthermore, zincuria is one of the symptoms of diabetes\(^11\)\(^,\)\(^12\), and zinc supplementation ameliorates glycemia in both type 1 and type 2 diabetes\(^13\)\(^–\)\(^15\). These observations show that understanding zinc homeostasis in health and disease is of importance. In the present review, we give an overview of the zinc transporters in the pancreatic \(\beta\)-cell with a focus on the recently discovered transporter, zinc transporter 8 (ZnT8), which has been associated with the development of both type 1 and type 2 diabetes.

EXPRESSION OF ZINC TRANSPORTERS IN ISLETS OF LANGERHANS

Our understanding of cellular zinc homeostasis has increased significantly with the discovery of a large group of zinc transporter proteins that allow diffusion of zinc ions across biological membranes\(^16\). These transporters are classified into two protein families (zinc transporter [ZnT] and Zrt/Irt-like protein [ZIP]), encoded respectively by *Slc30a*\(^1\)\(^,\)\(^4\), Slc39a\(^1\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\); Slc39a\(^8\)\(^,\)\(^9\)\(^,\)\(^11\)\(^,\)\(^13\)\(^,\)\(^14\)\(^,\)\(^20\) and Slc39a\(^1\)\(^2\)\(^,\)\(^14\)\(^,\)\(^20\)\(^,\)\(^21\)\(^,\)\(^22\). Because most tissues and organs express several ZnT and ZIP isoforms, the precise mechanism of zinc homeostasis in any cell type is a result of coordinated expression and activities of these zinc transporter isoforms. This interplay between transporters is further complemented with the distribution of zinc in the cell by metallothioneins (MT), of which four isoforms have been identified to date\(^20\). An overview of the messenger ribonucleic...
acid (mRNA) expression profile of the different zinc transporters (ZnT and ZIP) and metallothioneins (MT) in a panel of mouse tissues, measured by microarray using Affymetrix 430_2 arrays. Data are expressed as percent expression in a specific tissue, with the sum of the expression values of a specific transporter in all tissues set as 100%. The microarray data have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession nos. GSE24209 and GSE24940. ES cells, embryonic stem cells; ZIP, Slc39a zinc transporter family; ZnT, Slc30a zinc transporter family.

Figure 1 | Zinc transporter 8 (ZnT8) is specifically and abundantly expressed in islets of Langerhans. Messenger ribonucleic acid expression of the different zinc transporters (ZnT and ZIP) and metallothioneins (MT) in 22 different C57Bl6 mouse tissues, measured by microarray using Affymetrix 430_2 arrays. Data are expressed as percent expression in a specific tissue, with the sum of the expression values of a specific transporter in all tissues set as 100%. The microarray data have been submitted to the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession nos. GSE24209 and GSE24940. ES cells, embryonic stem cells; ZIP, Slc39a zinc transporter family; ZnT, Slc30a zinc transporter family.

Zinc transporters in the β-cell
be responsible for the uptake of zinc in synaptic vesicles of glutamatergic hippocampal neurons\textsuperscript{32}, and given that the β-cells have a neuron-like phenotype\textsuperscript{33,34}. The literature, however, is somewhat ambiguous for ZnT3 expression in β-cells; it was reported to be expressed in the insulinoma cell line INS1\textsuperscript{15}, but could not be confirmed in mouse islets\textsuperscript{36}. Although no detailed studies have been carried out to investigate the role of individual ZIP transporters in islet β-cells, it is clear that these follow a wide tissue distribution (Figure 1).

It can be assumed that most of the panel of ZnT and ZIP transporters with wide tissue distribution serve a housekeeping role in cellular zinc homeostasis, ensuring an equilibrium of zinc over the different subcellular compartments where the metal is required as a protein cofactor. An important question, however, is why ZnT8 is intensely and specifically expressed in islets. One possibility is that the turnover of ZnT8 mRNA and protein is very high, but this idea is not yet supported by experimental data. Another possibility is that high and specific expression of ZnT8 protects the β-cell from toxic effects of excess or shortage of zinc. As stated in the Introduction, mammalian pancreatic β-cells have one of the highest zinc concentrations of the whole organism. There is so much zinc in β-cells, that this property can be used to sort β-cells from islet preparations\textsuperscript{37}. As for most essential metals, a bell shape concentration dependency exists for cell viability, so that cells need to be protected against excess\textsuperscript{38} and depletion\textsuperscript{39} of zinc. The role of ZnT8 in β-cell apoptosis during zinc depletion was studied in INS-1E cells\textsuperscript{32}. Overexpression of ZnT8 protected the cells against apoptosis after treatment with the zinc chelator N,N,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN); in contrast, no effect on viability was reported during hyperzinc conditions. A third possibility, which will be discussed in the next section, is that the high expression of ZnT8 in β-cells is required for the process of regulated insulin synthesis, storage and secretion.

Regulation of the expression and localization of zinc transporters has been observed\textsuperscript{16}. Zinc availability in dietary intake regulates the expression of many zinc transporters, in particular isoforms of the ZiP family, and this regulation supports cellular zinc homeostasis. Phenotypic effects of ZIP knockouts can often be compensated by an increased dietary zinc or zinc supplementation, including mutations in the zinc transporter SLC39A4, mutations of which have been identified in acrodermatitis enteropathica, an autosomal recessive metabolic disorder affecting the uptake of zinc in the intestine\textsuperscript{40}. Recently, zinc transporters have been shown to be regulated by microRNA. Indeed, Slc39a5 (Zip5) translation is mediated by conserved elements in the 3′-untranslated region\textsuperscript{41}; in primary prostatic epithelial cells, the mir-183-96-182 cluster is overexpressed and regulates zinc homeostasis by suppressing mRNA levels of six zinc transporters (hZIP1, hZIP3, hZIP7, hZIP9, hZnT1, hZnT7)\textsuperscript{42}. In β-cells, it has been shown that glucose and cytokines play a role in the regulation of the expression of the zinc transporters. High glucose has no effect on the expression of ZnT8, but in contrast Zip6, Zip7 and Zip8 are upregulated in mouse islets cultured for 24 h on 16.7 mmol/L glucose\textsuperscript{36}. Under the same conditions, MT1 and MT2 are downregulated, and this decrease depends on elevations in cyclic adenosine monophosphate (cAMP). Cytokines (interleukin-1β [IL-1β], tumor necrosis factor [TNF-α]) decrease the expression of zinc transporters, particularly ZnT8\textsuperscript{43,44}. Expression of ZnT8 is also under the control of the transcription factor, pancreatic and duodenal homeobox 1 (PDX1), which regulates expression through two intronic enhancers, A and B\textsuperscript{45}. Enhancer A is a β-cell specific enhancer, whereas enhancer B promotes expression both in α- and β-cells.

**ROLE OF ZNT8 IN INSULIN PROCESSING AND STORAGE: FORMATION OF ZINC-INSULIN CRYSTALS**

β-Cells have the important physiological role of synthesizing, storing and secreting exactly the right amounts of insulin in order to meet the daily metabolic demands of the organism\textsuperscript{46}. Several studies have shown that zinc is essential for the different processes involved in the insulin secretory pathway\textsuperscript{47,48}. In the endoplasmic reticulum (ER), proinsulin folds with the help of chaperones into its 3-D structure with the formation of correct disulphide bonds; this folding is a prerequisite for the formation of proinsulin hexamers, which occurs in the Golgi apparatus\textsuperscript{49}. Two proinsulin dimers interact through their HisB10 residues with two zinc ions to form a proinsulin tetramer, which then combines with another dimeric unit to give the zinc\textsubscript{2} proinsulin\textsubscript{4} hexamer\textsuperscript{50}. After passing the Golgi apparatus, prohormone convertases (PC), PC1/3 and PC2, as well as carboxypeptidase E, convert zinc-proinsulin hexamers in immature secretory vesicles into zinc-insulin hexamers\textsuperscript{51}. This conversion, together with granular acidification\textsuperscript{52}, cause a profound change of the surface structure of the protein hexamers; in the presence of physiological zinc concentration, proinsulin hexamers are water soluble, whereas insulin hexamers are water insoluble\textsuperscript{53}. The consequence of these events is that zinc-insulin crystals are formed, so that the immature secretory granules transform into mature granules with a typical ultrastructural appearance of an electron-dense core surrounded by a halo (Figure 2a).

Because zinc is required for hexamerization and conversion of proinsulin to insulin\textsuperscript{46}, sufficient uptake of zinc ions in the ER and Golgi compartments is required. As explained in the previous section, possible candidates for this transport are ZnT5 and ZnT7, both shown to be present in the islets and expressed at the appropriate location of insulin biosynthesis and folding\textsuperscript{9,50}. In contrast, ZnT8 seems to not be required for conversion of proinsulin to insulin, as studies with whole-body ZnT8-deficient (ZnT8\textsuperscript{-/-}) mice showed no defect in insulin processing\textsuperscript{26,27}. This was measured both through \textsuperscript{35}S pulse/chase experiments\textsuperscript{26,27} and in situ peptidomics\textsuperscript{26}. A conflicting result, however, was reported in a model of β-cell specific ZnT8 knockout mouse, in which proinsulin conversion, measured through a proinsulin-specific immunoassay, is delayed\textsuperscript{25}. The reason for this discrepancy is not entirely clear, but it should be mentioned that in
contrast to the whole animal knockout models\textsuperscript{26,27}, expression of critical proteins for proinsulin conversion (PC2, carboxypeptidase E) was also decreased in these \( \beta \)-specific knockout islets\textsuperscript{23}.

In contrast, one study reported that patients carrying the at-risk (R325) allele of ZnT8 have impaired conversion of proinsulin into insulin\textsuperscript{54}.

More than 80 years ago, it was suggested that zinc plays a role in the crystallization of insulin\textsuperscript{55}. As aforementioned, X-ray diffraction analysis has shown that per insulin hexamer, two zinc ions are bound to the six HisB10 residues of the hexamer. Comeasurements of insulin and zinc release from glucose stimulated islets, however, suggest that more zinc ions are present in zinc-insulin crystals than the 2:6 ratio in the hexamers\textsuperscript{56}. Indeed, zinc release from glucose-stimulated islets was measured through a gadolinium-based zinc sensor, and was 1.73 ± 0.74 pmol/islet equivalent, whereas insulin release from the same islets was 2.65 ± 1.13 pmol/islet equivalent, resulting in a 4:6 zinc:insulin ratio instead of a 2:6 ratio. This shows that twofold more zinc is present in the dense core granules containing insulin crystals, compared with the hexamers, and it is conceivable that this extra zinc, which is required to displace water molecules between insulin-zinc hexamers, is delivered through ZnT8. This idea is also supported by our observations that \( \beta \)-cells in ZnT8\textsuperscript{−/−} islets are incompetent to form normal amounts of insulin-zinc crystals\textsuperscript{26}. The most direct evidence in this work was obtained by electron microscopy, showing an almost complete replacement of dense core granules by immature granules in which a dense core is lacking (Figure 2a). As in these mice insulin content per islet and proinsulin conversion were found to be normal, it can be assumed that the immature granules are filled with water-soluble insulin. Another piece of evidence is lack of detectable release of zinc on exocytosis of insulin granules, whereas insulin release occurs normally\textsuperscript{26}. This dissociation again shows that more zinc ions are bound to insulin in the crystal in addition to the two ions bound per hexamer. Because of the normal insulin processing, which normally occurs in the presence of zinc in proinsulin hexamers, and the normal expression of the other ZnT transporters, we presume that ZnT8\textsuperscript{−/−} islets store insulin as zinc:insulin hexamers\textsuperscript{26}. However, if this is the case, it is not clear why the ZnT8\textsuperscript{−/−} islets lack detectable zinc release completely, as the crystal would only have twofold more zinc, based on the aforementioned calculations. Is this because zincinsulin hexamers are not readily dissolved on release like insulin crystals and therefore not detected with the applied technique, or is zinc reabsorbed from the mature secretory granules before exocytosis? Unfortunately, answering these questions awaits further analysis. Hence, the \( \beta \)-cell of ZnT8\textsuperscript{−/−} mice has a biophysical phenotype in which the physical form of stored insulin is changed from crystalline to water soluble. This is also reflected in striking changes\textsuperscript{26} in familiar aspects of islets of

\begin{figure}[h]
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\caption{Zinc transporter 8 (ZnT8) is required for zincinsulin crystallization. (a) Absence of typical insulin-dense core granules in ZnT8 (ZnT8\textsuperscript{−/−}) deficient mice, seen by transmission electron microscopy. Absence of dithizone-stained islets in ZnT8\textsuperscript{−/−} mice (b) \textit{in situ}, as well as (c) \textit{in vitro}. (d) Bright light reflection of isolated wild-type (ZnT8\textsuperscript{+/+}) islets compared with the more transparent appearance of ZnT8\textsuperscript{−/−} islets, which lack zincinsulin crystals. (e) A somewhat analogous situation occurs when comparing white light reflection of small sugar crystals vs transparent sugar solution. Figures are reproduced or adapted from Lemaire et al.\textsuperscript{26} with permission.}
\end{figure}
Langerhans, namely the typical staining with the dye dithizone (Figure 2b,c)57 and the bright scattering of light (Figure 2d,e). Osmosis is another consequence of the change in storage form, because soluble insulin acts as an osmolite. It can be predicted that for the same amount of stored molecules, water-soluble insulin would require more space. Measurements of granule diameter in wild-type and ZnT8−/− mice have shown that insulin-zinc crystals require approximately 30% less space than water-soluble insulin26.

Besides a more compact packaging of insulin in the secretory granules, zinc-insulin crystallization is also believed to protect insulin against proteolytic degradation58,59. However, several data argue against this idea: (i) the presence of an intact 5808 Da insulin peptide as one sharp mass peak in the spectrum of islets of both wild-type and ZnT8−/− mice, measured by in situ peptidomics26; and (ii) the non-crystallized guinea pig and coypu insulin would be more vulnerable to degradation, compared with human crystallized insulin60,61.

ARE ZINC-INSULIN CRYSTALS REQUIRED FOR NORMAL GLUCOSE HOMEOSTASIS?

Nature has allowed variations on the theme that was discussed in the last section. In several vertebrates, such as hagfish, guinea pig and coypu, variation in the insulin gene sequence causes a structural change in the insulin B-chain, so that HisB10 is lacking62. In these species, proinsulin does not associate with dimers and hexamers, and consequently no zinc-insulin crystals are formed63. Consistent with the predicted consequence, the secretory granules of guinea pig β-cells do not contain detectable zinc64. A second variation of nature is a mutant human insulin gene in which the codon encoding HisB10 is replaced by an aspartic acid codon (His-B10-Asp). With this mutation, the β-cells lose the capacity to form normal dense core vesicles65. Besides pale, immature secretory vesicles, vesicles containing a rod-shaped dense core are also present. These patients are borderline glucose intolerant and hyperproinsulinemic66. Over-expression of the mutant insulin gene in mice results in normoglycemic mice65. In ZnT8−/− mice in which insulin crystal formation is impaired, the consequences of glucose homeostasis vary between studied strains. In our own study, where β-cells contained mainly pale vesicles, we observed normal fasting blood glucose levels, glucose tolerance and glucose-stimulated insulin release under normal diet conditions26. However, in other strains of ZnT8−/− mice studied in parallel in London and Toronto, a mix of rod-shaped, pale and dense core secretory vesicles were present, and relatively small abnormalities in glucose tolerance were reported27. The biological difference between normal dense-core, rod-shaped and pale vesicles is interesting, but incompletely understood. It is conceivable that the spherical geometric positioning of ZnT8 molecules in the granule membrane around the granule core influences the free zinc concentrations in the water space around the crystal, and hence the flux of new zinc molecules from the water space to the crystal (Figure 3). Together, these factors would favor spherical symmetry in the displacement of water molecules by zinc and the growth of a spherical dense core. It is also possible that, depending on the expression of other zinc transporters in the granule membrane in the different mouse strains, different amounts of zinc are still present in the vesicles allowing growth of abnormal crystals (rods or plates), which are predicted to be less efficient in terms of packaging efficiency in granules, and perhaps even harmful for the integrity of the granule membrane. Thus, it is possible that, depending on the kinetics of zinc influx from the cytosol to the granular lumen and the rate of zinc incorporation into new crystal, the shape of the crystal varies between spherical and rod-like, whereas in the complete absence of transporters, no crystal is formed (Figure 3). Detailed real-time measurements of free zinc in maturing granules would be required to resolve this important issue. Apart from differences between mouse strains, there could also be an effect of age, as secretory granules with a rod-shaped core predominate in young mice27, whereas the islets of older mice contain mainly immature vesicles without a dense core26,27. The environmental influence of diet might also play a role, as we observed a progressive deterioration of glucose homeostasis with time in ZnT8−/− mice fed a high-fat diet26. This deterioration is a result of abnormal β-cell function, as insulin sensitivity remained normal, whereas glucose-stimulated insulin release from isolated islets diminished. The effect of high-fat diet on insulin secretory granules was not analyzed, but it is conceivable that impaired granule packaging in β-cells that cannot form insulin crystals leads to secretory failure when the metabolic insulin demand is high.

In human pharmacology, water-soluble (rapidly acting) insulin reaches the circulation at an earlier time-point than crystalline insulin preparations67. However, it is not known how fast secreted insulin crystals dissolve in the extracellular space. The diameter of the pore resulting from a ‘kiss and run’ event of exocytosis was estimated in β-cells to vary between 1.5 and 6 nm (human–mouse, respectively), which is smaller than the diameter of the dense core68. As a result, one could imagine that insulin, when stored in a non-crystallized water-soluble manner, such as what occurs in ZnT8−/− mice, would more easily be secreted during a ‘kiss and run’ event than insulin crystals from dense core granules. As insulin release (both time kinetics and percent released per β-cell per hour) is well preserved in the absence of insulin crystals26, it is clear that dissolving insulin crystals in the islet interstitium does not delay the rate at which insulin enters the circulation in vivo, at least not to the extent that this can be measured. Furthermore, Nicolson et al.27 also showed through total internal reflection fluorescence (TIRF) that no differences in the number of ‘full’ or ‘partial’ events were observed between the wild-type and whole-body ZnT8−/− mice. Together, these data show that insulin crystallization per se is
not essential for the dynamics of insulin release and normal glucose homeostasis.

**ZNT8 AND α-CELLS**

At the mRNA level, high amounts of ZnT8 expression were also found in mouse cells. However ZnT8 immunoreactivity is weak in α-cells as compared with β-cells, suggesting that gene expression in α-cells is inhibited at a post-translational step, or that protein stability is less in these cells than in β-cells.

The existence of paracrine effects within an intact islet of Langerhans has been documented by many studies. One important physiological regulation is that α-cells are suppressed under conditions that β-cells are active, and over the past decades, insulin, gamma aminobutaric acid (GABA) and zinc have been proposed as important paracrine β-cell factors that inhibit α-cells. The role of zinc, first proposed by Ishihara et al., is, however, a subject of intense debate. A detailed overview of the role of zinc on glucagon secretion can be found in recently-published reviews. Novel evidence was collected by the study of ZnT8−/− mice, which are deficient in zinc release; in these animals, no difference in plasma glucagon levels was observed, and glucagon release from isolated islets was the same as in control islets, suggesting that zinc secreted from β-cells does not regulate glucagon secretion.

**POSSIBLE ROLE OF ZNT8 IN THE ONSET OF DIABETES**

As was already mentioned in the Introduction, a link between diabetes and zinc homeostasis was first noted in 1934. At the genetic level, this link was recently further supported by several genome-wide association studies that reported a point mutation in the ZnT8 gene, leading to an Arg325Trp polymorphism, with genetic susceptibility to type 2 diabetes. A meta-analysis, including 32 cohorts, showed that each ZnT8 risk C-allele (Arg-isoform) was associated with a 14% increased risk for type 2 diabetes. The presence of the risk allele, however, had no effect on basal or stimulated ex vivo insulin secretion or on ZnT8, insulin and glucagon expression. In contrast, ZnT8 expression was positively correlated with circulating insulin and glucagon levels. In vitro experiments carried out with INS1 cells overexpressing the Arg- or Trp-isoform of ZnT8 showed no difference in glucose-stimulated insulin release or membrane potential, but a markedly decreased zinc uptake was observed when the risk Arg-isoform was overexpressed. Therefore, the exact mechanistic role of the Arg325Trp polymorphism in the predisposition for diabetes is unclear at the moment. The reason for the discrepancy between the predisposition for diabetes in humans and the lack of a drastic effect on glucose homeostasis in ZnT8−/− mice is unclear. A first possibility is that ZnT8...
function in mouse and human β-cells is not completely the same, analogous to what was proposed for facilitative glucose transporters. Alternatively, the epistatic effect of an unidentified extra genetic factor could be required for the increased susceptibility in the human locus. Third, the observed genetic susceptibility of the locus could be unrelated to ZnT8, one example being a regulatory element belonging to a neighboring gene.

The ZnT8 gene and its encoded protein could be linked to diabetes through other mechanisms than the Arg325Trp polymorphism. First, disturbances in zinc homeostasis and increased levels of oxidative stress play a major role in the pathogenesis of diabetes, both type 1 and type 2. Zinc depletion by itself can induce apoptosis, but can also promote oxidative stress-induced apoptosis, resulting in reduced β-cell mass. Although zinc is redox inert, and therefore in itself not an antioxidant, it shows a variety of indirect anti-oxidant effects, thus explaining its role in oxidative stress-induced apoptosis and diabetes. Therefore, increasing the capacity of the β-cell to store zinc – for example, overexpression of ZnT8 or MT – could protect the pancreas against zinc depletion and oxidative stress often observed in diabetes. In favor of this idea, a human MT1A polymorphism has been linked with the development of type 2 diabetes. Furthermore, a single nucleotide polymorphism (SNP) in the 3′ untranslated region of ZnT8, which is correlated with increased fasting glucose levels, has been identified, and an inverse association between total zinc intake and fasting glucose was observed in individuals carrying the glucose-raising allele.

Finally, ZnT8 was observed to be a major autoantigen in human type 1 diabetes and a predictive marker in risk groups before onset of the disease. Wenzlau et al. showed that ZnT8 was targeted by autoantibodies in 60–80% of new-onset type 1 diabetic patients, as compared with <2% of control subjects. Including anti-ZnT8 antibodies to the list of already-used predictive markers, Glutamic acid decarboxylase antibodies (GADA), tyrosine phosphatase antibodies (IA2A), insulin autoantibodies (IAA) and islet cell antibodies (ICA), raised autoimmunity detection rates to 98% at disease onset.

PERSPECTIVE
Together, the analysis of ZnT8 in normal health and diabetes has so far yielded an incomplete image with many gaps that need to be filled by further study. On the one hand, the protein is required for insulin crystal formation, but this process is not essential for normal glucose homeostasis. On the other hand, a genetic polymorphism in the ZnT8 gene predisposes to type 2 diabetes, but the underlying mechanism is unclear. In fact, there is a discrepancy between the effect of a ZnT8 polymorphism and human diabetes, and the lack of diabetes in mice in which ZnT8 is not expressed. In order to better understand this discrepancy, it might be necessary to make a knock-in mouse model that recapitulates the human risk allelic variant. In this way, we will be able to analyze if a change in the encoded protein or an altered regulatory element for a neighboring gene is responsible for the increased susceptibility. Finally, it is clear from many studies that zinc is essential for β-cell function. This has very recently been shown in a study of intraportal islet transplantation in rats that was more successful in a zinc-rich environment. Together, all available evidence strongly shows that it is important to further study the factors that regulate zinc homeostasis in β-cells and the mechanisms that lead to failure of this homeostasis and β-cell dysfunction.

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