

Zinc transporters and their role in the pancreatic β -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 β -cell contains the highest zinc content of any known cell type, it is of interest to know how zinc fluxes are controlled in β -cells. The understanding of zinc homeostasis has been boosted by the discovery of multiprotein 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 cross-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 β -cells and how abnormalities lead to β -cell dysfunction and diabetes. (*J Diabetes Invest*, doi: 10.1111/j.2040-1124.2012.00199.x, 2012)

KEY WORDS: Insulin crystallization, Pancreatic β -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, proliferation, differentiation and apoptosis^{1–3}. 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 β -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 vesicles⁴. 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 population⁷. 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 year⁸. 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 β -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 membranes¹⁶. These transporters are classified into two protein families (zinc transporter [ZnT] and Zrt/Irt-like protein [ZIP]), encoded respectively by *Slc30a* and *Slc39a* genes that have multiplied during eukaryotic evolution by gene duplication events¹⁷. The mammalian ZnT protein family comprises 10 members and they transport zinc out of the cytosol, both into the extracellular space and into the compartments of cellular organelles. This opposes the direction of zinc flux mediated by members of the ZIP family (14 isoforms known in mammals), which transport zinc back into the cytosol^{16,18,19}. 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 date²⁰. An overview of the messenger ribonucleic

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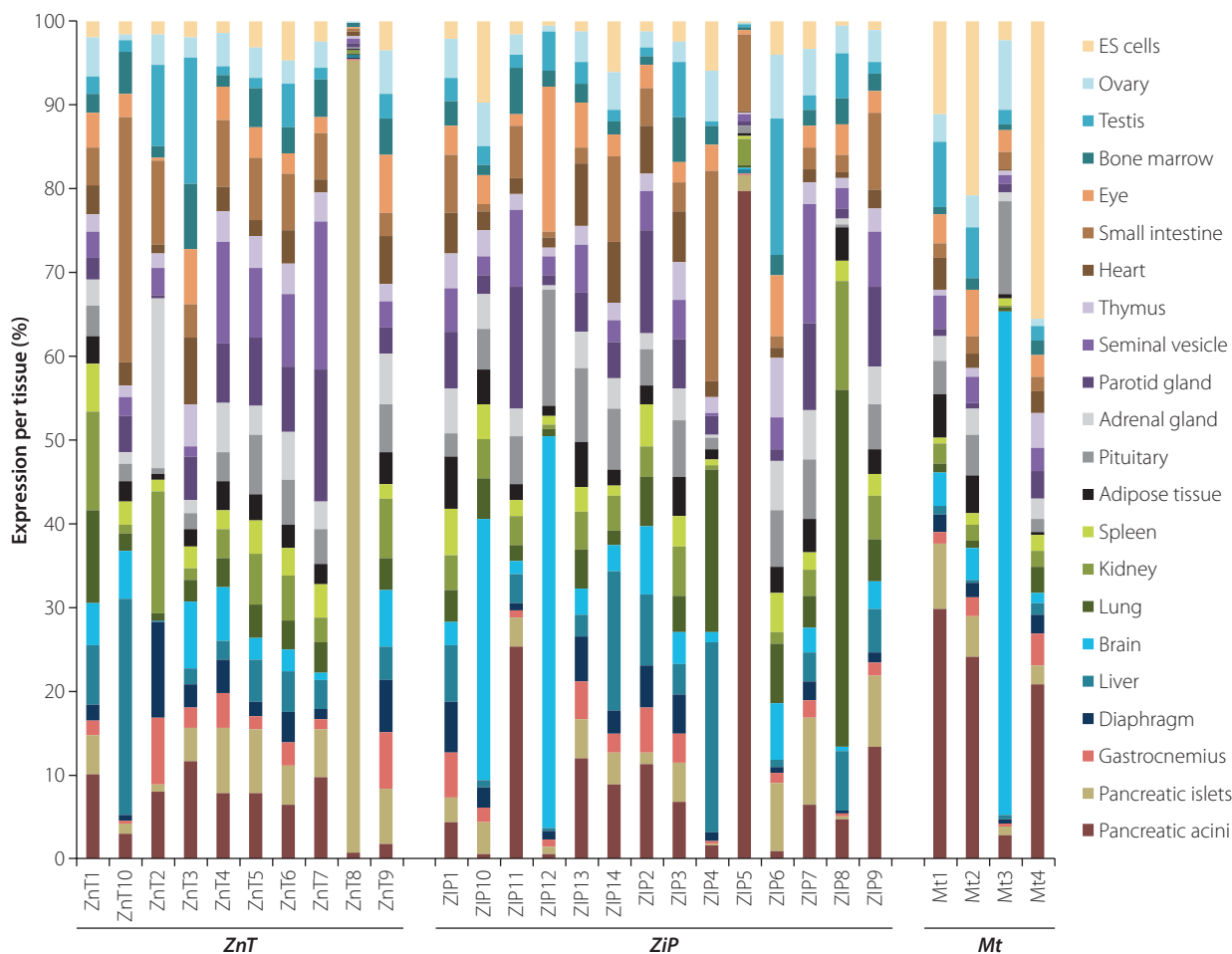


Figure 1 | Zinc transporter 8 (*ZnT8*) is specifically and abundantly expressed in islets of Langerhans. Messenger ribonucleic acid expression of the different zinc transporters (*ZnT* and *ZIP*) and metallothioneins (*Mt*) in 22 different C57Bl6 mouse tissues, measured by microarray using Affymetrix 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.

acid (mRNA) expression profile of the different zinc transporters and *MT* in a panel of mouse tissues and organs is shown in Figure 1. Pancreatic islets of Langerhans do not form an exception in the sense that various *MT*, *ZnT* and *ZIP* isoforms are expressed together (Figure 1). However, the mRNA expression profile of *ZnT8* is exceptional in two ways. First, as can be seen in Figure 1, this is the only transporter with an outspoken tissue-specific expression, being detectable in pancreatic islets only and giving background signals in the other 21 examined mouse tissues. Second, the expression level is highest of all isoforms and comparable with the expression of β -actin. This makes *ZnT8* by far the most expressed transporter in islets of Langerhans. Pioneering studies by Chimienti *et al.* showed that *ZnT8* is primarily expressed in secretory granules of islet β - and α -cell^{21,22}, whereas its presence in the δ - and pancreatic polypeptide (PP)-cells is still under debate^{21–25}. *ZnT8* is mainly present as a homodimer

of ~ 70 kDa^{26,27}. The 3-D structure of *ZnT8* dimers is based on the *Escherichia coli* zinc transporter, YiiP transporter^{27,28}, and consists of parallel oriented monomers held together through four zinc ions at the interface of the cytoplasmic domains, with two transmembrane domains swinging out to yield a Y-shaped structure. Based on *in silico* mutagenesis, this specific conformation is similar in the different *ZnT8* alleles, as described later²⁷. Besides *ZnT8*, *ZnT5* and *ZnT7* are also reported to be expressed in the β -cell^{29,30}, but these transporters are expressed in other tissues as well (Figure 1). *ZnT5* co-localizes with the Golgi apparatus and secretory vesicles, whereas *ZnT7* resides mostly in the perinuclear region. In chicken DT40 cells, *ZnT5* forms functional heterodimers with *ZnT6*³¹, which might be a rationale for our observation that *ZnT6* is also expressed in islets (Figure 1). The role of *ZnT3* in β -cells received some attention in the literature, because this isoform has been proposed to

be responsible for the uptake of zinc in synaptic vesicles of glutaminergic hippocampal neurons³², and given the fact that β -cells have a neuron-like phenotype^{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 INS1³⁵, but could not be confirmed in mouse islets³⁶. 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 preparations³⁷. As for most essential metals, a bell shape concentration dependency exists for cell viability, so that cells need to be protected against excess³⁸ and depletion³⁹ of zinc. The role of ZnT8 in β -cell apoptosis during zinc depletion was studied in INS-1E cells²². 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 observed¹⁶. 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 enteropatica, an autosomal recessive metabolic disorder affecting the uptake of zinc in the intestine⁴⁰. Recently, zinc transporters have been shown to be regulated by microRNA. Indeed, *Slc39a5* (*Zip5*) translation is mediated by conserved elements in the 3'-untranslated region⁴¹; 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)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 glucose³⁶. Under the same conditions, *MT1* and *MT2* are downregulated, and this decrease depends on elevations in cyclic adenosine monophosphate (cAMP). Cytokines (interleukin-1 β [IL-1 β], tumor necrosis factor [TNF- α]) decrease the expression of zinc transporters, particularly *ZnT8*^{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 B⁴⁵. 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 organism⁴⁶. Several studies have shown that zinc is essential for the different processes involved in the insulin secretory pathway^{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 apparatus⁴⁹. 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 zinc₂ proinsulin₆ hexamer⁵⁰. 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 hexamers⁵¹. This conversion, together with granular acidification⁵², 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 insoluble⁵³. 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 insulin⁴⁹, 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^{9,30}. In contrast, ZnT8 seems to not be required for conversion of proinsulin to insulin, as studies with whole-body ZnT8-deficient (*ZnT8*^{-/-}) mice showed no defect in insulin processing^{26,27}. This was measured both through ³⁵S pulse/chase experiments^{26,27} and *in situ* peptidomics²⁶. 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 delayed²³. The reason for this discrepancy is not entirely clear, but it should be mentioned that in

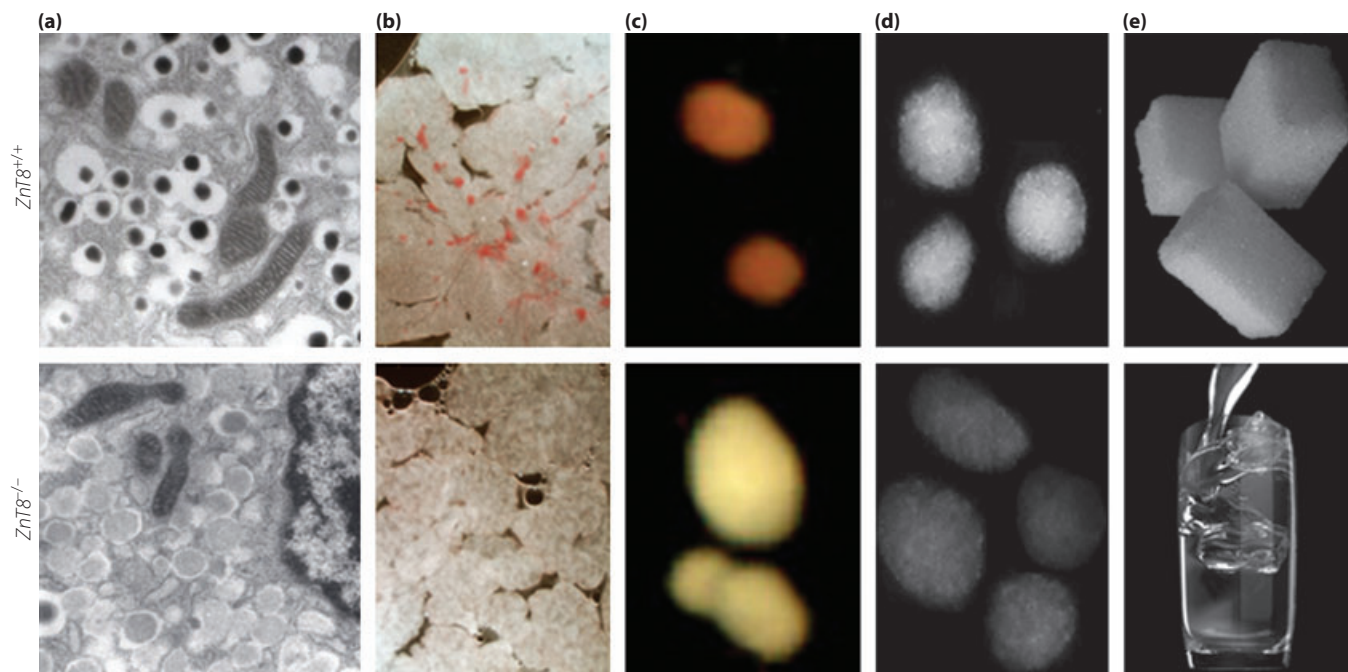


Figure 2 | Zinc transporter 8 (ZnT8) is required for zinc:insulin crystallization. (a) Absence of typical insulin-dense core granules in ZnT8 ($ZnT8^{-/-}$) deficient mice, seen by transmission electron microscopy. Absence of dithizone-stained islets in $ZnT8^{-/-}$ mice (b) *in situ*, as well as (c) *in vitro*. (d) Bright light reflection of isolated wild-type ($ZnT8^{+/+}$) islets compared with the more transparent appearance of $ZnT8^{-/-}$ islets, which lack zinc:insulin 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.*²⁶ with permission.

contrast to the whole animal knockout models^{26,27}, expression of critical proteins for proinsulin conversion (PC2, carboxypeptidase E) was also decreased in these β -specific knockout islets²³. In contrast, one study reported that patients carrying the at-risk (R325) allele of ZnT8 have impaired conversion of proinsulin into insulin⁵⁴.

More than 80 years ago, it was suggested that zinc plays a role in the crystallization of insulin⁵⁵. 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 hexamers⁵⁶. 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 β -cells in $ZnT8^{-/-}$ islets are incompetent to form normal amounts of insulin-zinc crystals²⁶. 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 normally²⁶. 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 $ZnT8^{-/-}$ islets store insulin as zinc:insulin hexamers²⁶. However, if this is the case, it is not clear why the $ZnT8^{-/-}$ islets lack detectable zinc release completely, as the crystal would only have twofold more zinc, based on the aforementioned calculations. Is this because zinc:insulin 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 β -cell of $ZnT8^{-/-}$ 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 changes²⁶ in familiar aspects of islets of

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 osmolyte. 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 degradation^{58,59}. 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 insulin^{60,61}.

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. Overexpression 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 core^{26,27}. 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

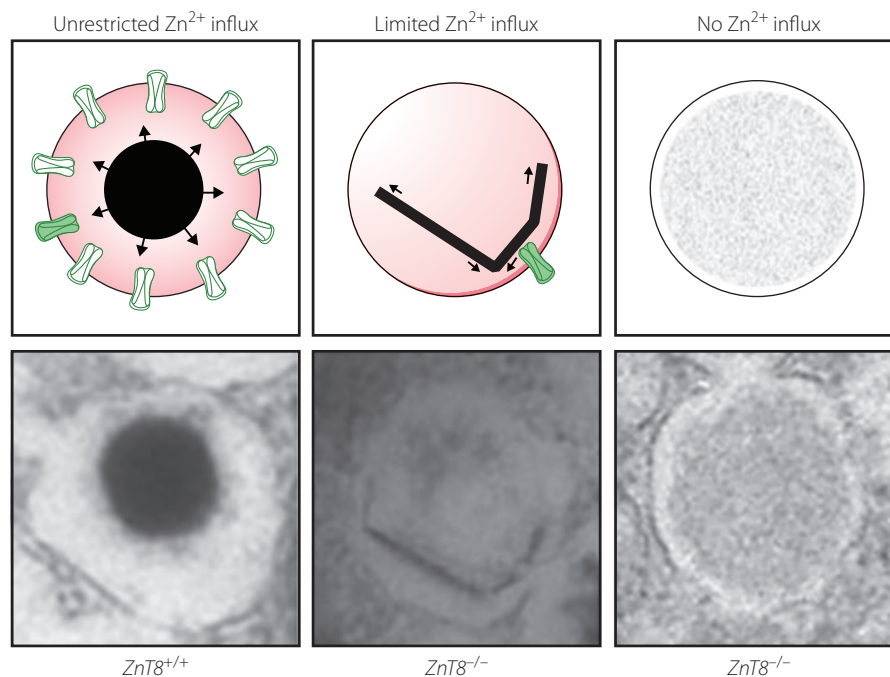


Figure 3 | Hypothetical model for the formation of insulin crystals in secretory vesicles. (a) In the presence of zinc transporter 8 (ZnT8), zinc is equally and rapidly transported into the water space around the crystal in the secretory vesicle, so that transport does not limit the symmetry of crystal formation and the granule-dense core grows in a spherical manner. However, when zinc influx is hampered, for example, because of the absence of ZnT8, crystal formation will be affected. (b) One possibility is asymmetric influx of zinc, which could cause the growth of rod-shaped crystals. (c) Another possibility is absence of zinc transporters, which could result in the lack of crystals and a secretory granule filled with water-soluble insulin. Upper panels: white transporter, ZnT8; green transporter, unidentified ZnT transporter; black surface, insulin crystal; black arrows, growth direction of the crystal. Lower panels: detailed electron microscopy view of a secretory granule of an islet of the $ZnT8^{+/+}$ and $ZnT8^{-/-}$ mouse.

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^{26,27}. However ZnT8 immunoreactivity is weak in α -cells as compared with β -cells²⁶, 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^{69–72}. One important physiological regulation is that α -cells are suppressed under conditions that β -cells are active⁷³, and over the past decades, insulin⁷⁴, gamma aminobutyric acid (GABA)⁷⁵ and zinc⁶⁹ have been proposed as important paracrine β -cell factors that inhibit α -cells. The role of zinc, first proposed by Ishihara *et al.*⁶⁹, is, however, a subject of intense debate^{70,76,77}. A detailed overview of the role of zinc on glucagon secretion can be found in recently-published reviews^{76,77}. Novel evidence was collected by the study of $ZnT8^{-/-}$ mice, which are deficient in zinc release²⁶; in these animals, no difference in plasma glucagon levels was observed, and glucagon release from isolated islets was the same as in control islets^{26,27,77}, 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^{55,78}. 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^{54,79–84}. 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 diabetes⁸⁵. 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 overexpressed²⁷. 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 transporters⁸⁶. 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^{13,87}. Zinc depletion by itself can induce apoptosis³⁹, but can also promote oxidative stress-induced apoptosis⁸⁸, resulting in reduced β -cell mass. Although zinc is redox inert, and therefore in itself not an anti-oxidant, it shows a variety of indirect anti-oxidant effects⁹, 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 diabetes⁸⁹. Furthermore, a single nucleotide polymorphism (SNP) in the 3' untranslated region of *ZnT8*, which is correlated with increased fasting glucose levels, has been identified⁹⁰, 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^{91,92}. 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 subjects⁹¹. 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 environment⁹³. 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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