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Zinc, the pancreas, and diabetes: Insights from rodent studies and future directions

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Abstract

Molecular and cellular studies have demonstrated several roles for zinc (Zn) in insulin production and the consequent actions of insulin on metabolism. Clinical and epidemiological studies suggest that reduced Zn status is associated with diabetes. Investigations of Zn in rodent models of diabetes have provided a valuable link for understanding the molecular, cellular, clinical and epidemiological observations in the context of inter-organ metabolism and the metabolic disturbances of diabetes. This review highlights some of the current knowledge and future research directions for the role of Zn in the pancreas and diabetes based on rodent studies and experimental manipulations of Zn. Overall, Zn supplementation is effective for preventing or ameliorating diabetes in several rodent models of Type 1 and Type 2 diabetes. Studies with chemically-induced Type 1 diabetes indicate that the protective effects of Zn involve antioxidant mechanisms whether it is Zn alone (as an antioxidant), Zn induction of metallothionein or Zn inhibition of redox-sensitive transcription factors. Further studies are needed to identify the mechanism(s) for Zn protection in Type 2 diabetes, including pancreatic and peripheral effects. Experimental manipulations of Zn status in rodent models of diabetes provide a valuable approach to explore mechanisms for the protective effects of Zn; however, long term clinical studies establishing safety (lack of toxicity) and efficacy are required before any recommendations can be made for people with diabetes.

Abbreviations: GK rats – Goto-Kakizaki rats; MT – metallothionein; STZ – streptozotocin; Zn – zinc

Introduction

At the molecular and cellular level, zinc (Zn) is intimately involved in insulin synthesis, secretion and signalling, and thus, the consequent actions of insulin on metabolism (reviewed by Chausmer 1998; Tallman & Taylor 1999). Various clinical and epidemiological studies suggest that reduced Zn status is associated with diabetes (reviewed by Chausmer 1998; Tallman & Taylor 1999). Manipulations of Zn status in rodent models of diabetes have been an important experimental approach to link what is known at the molecular and cellular levels with the clinical and epidemiological observations. Rodent models are amenable to manipulation of Zn status through various approaches (e.g. dietary Zn level, oral Zn supplementation, Zn injections) and allow for *in vivo* investigation of Zn in the context of inter-organ metabolism and the metabolic disturbances of diabetes. The objective of this review is to highlight some of the current knowledge and future research directions for the role of Zn in the pancreas and diabetes based on rodent studies and experimental manipulations of Zn.

Zn and the pancreas

The pancreas is the site of insulin synthesis, storage and secretion, and Zn is involved in each of these processes (Dodson & Steiner 1998). Zn ions bind insulin in a hexameric crystalline structure which is stored in secretory granules. Studies with ^{65}Zn have shown that secretory granules contain one-third of islet Zn (Figlewicz *et al.* 1984). Several techniques have been used to visualize Zn in subcellular compartments of pancreatic islets (e.g. X-ray microprobe analysis (Falkmer *et al.* 1985), fluorescent probes for Zn (Zalewski *et al.* 1994), autometallography (Kristiansen *et al.* 2001)). These techniques could be used for comparison of pancreatic Zn localization with presence of diabetes and changes in Zn status (deficient versus supplemented). To date, Sondergaard *et al.* (2003) has reported no differences in the ultrastructural localization of Zn ions in secretory granules among *fa*/*fa* Zucker rats (insulin-resistant and obese model), Goto-Kakizaki (GK) rats (non-obese Type 2 diabetes model) and their respective controls during steady-state glycemia, using autometallography.

There is a complex signalling cascade in pancreatic islets for glucose-stimulated insulin secretion that involves ATP-sensitive potassium (K_{ATP}) channels (Rutter 2004). In the presence of glucose, an increase in the intracellular ATP/ADP ratio closes the K_{ATP} channels resulting in depolarization of the plasma membrane, influx of extracellular calcium and activation of exocytosis. The islet cell plasma membrane has K_{ATP} channels, however, the majority of K_{ATP} channels are localized on secretory granule membranes (Geng *et al.* 2003). The pancreatic K_{ATP} channels have four regulatory sulfonylurea receptor (SUR1) subunits and four potassium pore-forming ($K_{ir}6.2$) subunits. Recent studies identify Zn as an intracellular and extracellular regulator of K_{ATP} channels (Prost *et al.* 2004) and that Zn binding to two histidine residues (H326 and H332) on the SUR1 subunit activates the K_{ATP} channels (Bancila *et al.* 2004). The next step will be to determine whether functioning of K_{ATP} channels is responsive to *in vivo* alterations in plasma and pancreatic Zn concentrations due to dietary Zn intake or diabetes status.

Exocytosis of secretory granules releases insulin and Zn (Qian *et al.* 2003). Based on perfusion studies with isolated pancreata, Ishihara *et al.* (2003) are proposing that Zn released from β-cells during insulin secretion may be involved in paracrine communication for coordinated inhibition of glucagon secretion by α-cells. This coordinated response requires further investigation in the context of whole body metabolism.

The pancreas is a location of high Zn turnover and is one of the few tissues that shows reduced Zn concentration during Zn deficiency (Roth & Kirchgessner 1980). The observation by Scott & Fisher (1938) of a 50% reduction in pancreatic Zn concentration of diabetic cadavers compared to non-diabetic cadavers focussed attention on relationships among Zn, pancreas and diabetes. Similarly, reduced pancreatic Zn concentrations have been reported in genetic mouse models of Type 2 diabetes, *ob*/*ob* (mutation in *ob* (leptin) gene) and *db*/*db* (mutation in leptin receptor) mice (Begin-Heick *et al.* 1985; Kennedy & Failla 1987; Southon *et al.* 1988; Simon & Taylor 2001), and GK rats (Type 2 model produced by selective breeding of rats with glucose intolerance)(Takita *et al.* 2004) compared to non-diabetic controls. An exception is 20–25% higher pancreatic Zn concentrations in BB Wistar rats, a spontaneous autoimmune model of Type 1 diabetes, compared to non-diabetes prone Wistar rats (Failla & Gardell 1985; Tobia *et al.* 1998). Although attention has been placed on Zn and the pancreas, the consequences of reduced pancreatic concentrations of other minerals (e.g. Fe, Mn and Mg in GK rats, Takita *et al.* 2004) in consort with Zn has not been explored.

Isotope studies in rodents with ^{65}Zn have been valuable for characterizing whole-body Zn flux. The pancreas, along with intestine, liver, kidney and spleen, are characterized by high rates of Zn flux (Sheline *et al.* 1943). Within the pancreas, the acinar cells have higher rates of ^{65}Zn turnover compared to β-cells (McIsaac *et al.* 1955). This is explained by the high levels of Zn in exocrine secretions and their importance in regulating Zn homeostasis (De Lisle *et al.* 1996). Zn is critically involved in both exocrine and endocrine functions of the pancreas.

The identification of membrane proteins for Zn transport, the ZIP and Zn transporter (ZnT) families (Liuzzi & Cousins 2004), is revealing more about the cellular movement of Zn within tissues including the pancreas. Intracellular uptake of Zn

is regulated by ZIP proteins while cellular efflux of Zn into extracellular matrix or into intracellular vesicles is controlled by ZnT proteins (Liuzzi & Cousins 2004). Several ZIP and ZnT family members have been identified (e.g. 15 ZIP and 9 ZnT) which have specificity for different cell types and sub-cellular localization. A total of 16 ZIP and ZnT transporters are present in mouse pancreas with highest abundance for the cellular Zn exporters, ZnT1 and ZnT2 (Liuzzi *et al*. 2004). ZIP4 is present in β-cells while ZIP5 is expressed in acinar cells (Dufner-Beattie 2004). ZnT5 (expressed in most tissues) is abundant in islet cells, specifically the Golgi apparatus and secretory granules (Kambe *et al*. 2002), the sites for assembly and storage of the Zn-insulin complex. ZnT8 is specific to pancreas islet cells where it co-localizes with insulin in the secretory vesicles (Chimienti *et al*. 2004). ZnT8 facilitates accumulation of Zn from cytoplasm into intracellular vesicles; both ZnT8 and the Zn concentration in the culture medium determine the level of Zn accumulation (Chimienti *et al*. 2004). The presence of these membrane transporters for Zn raises interesting questions about their response to altered Zn status and presence of diabetes. In mice, 21 d Zn depletion reduced ZnT1 and ZnT2 mRNAs (primarily expressed in acinar tissue) $\sim$10-fold while other ZnTs and ZIPs present in islets were not altered by dietary Zn deficiency (Liuzzi *et al*. 2004). In pancreatic islets of Wistar BB rats (spontaneous, autoimmune model for Type 1 diabetes), mRNA levels of several ZnTs demonstrated developmental regulation but they were unchanged compared to non-diabetic control rats (Clifford & MacDonald 2000). Further studies are needed to establish whether ZIP and ZnT proteins are changed in other models of diabetes in combination with altered Zn status.

Adequate levels of pancreatic Zn may also be critical for providing antioxidant protection given that oxidative stress is a component of tissue damage in Type 1 and Type 2 diabetes and its associated complications (Reviewed by Ho & Bray 1999 and Robertson *et al*. 2004). Compared to several other tissues, β-cells have lower levels of antioxidant defense components and are susceptible to oxidative damage (Lenzen *et al*. 1996; Tiedge *et al*. 1997). Zn contributes to antioxidant defense as a component of CuZnSuperoxide dismutase (CuZnSOD) and metallothionein (MT). Transgenic mice overexpressing CuZnSOD are resistant to developing streptozotocin (STZ)- or alloxan-induced diabetes (Kubisch *et al*. 1994, 1997) as are mice with overexpression of MT in pancreatic β-cells (Chen *et al*. 2001). Thus, additional antioxidant protection has been proposed as a means for prevention and management of diabetes.

Zn and Type 1 diabetes models

Chemically-induced experimental rodent models for Type 1 diabetes utilize STZ or alloxan to destroy β-cells resulting in hypoinsulinemia; protocols may use one or two high dose injections to produce hyperglycemia rapidly (within 2–3 days), or a low dose multiple injection regimen that results in insulitis (immune component) along with the hyperglycemia (Like & Rossini 1976). Pretreatment with Zn injections (10 mg Zn/kg body weight subcutaneously 12 h before STZ injection) partially prevented hyperglycemia and development of diabetes in STZ rats (Yang & Cherian 1994). The Zn protection was attributed to Zn induction of MT, a scavenger of oxygen free radicals, and was associated with reduction of lipid peroxidation in pancreas and liver. SOD did not play a role in the protection as pancreatic and hepatic SOD activity was reduced in STZ rats and not altered by Zn injection. However, some of the protective effects of Zn treatment may be independent of MT as a low dose Zn pre-treatment (1 mg Zn/kg body weight) ameliorated STZ-induced diabetes in MT-null mice but had no effect in wild-type mice (Apostolova *et al*. 1997). The wild-type STZ mice were protected with high dose Zn (10 mg Zn/kg body weight) suggesting that the mechanism(s) for Zn protection are dose dependent.

Other studies have focussed on oral Zn (supplementation in diet or drinking water) and signalling mechanisms for the protective effects of Zn in different models of Type 1 diabetes. Dietary Zn supplementation (1000 mg Zn/kg diet versus 50 mg Zn/kg in the control diet) from age 30 to 100 days old delayed the onset and reduced severity of spontaneous diabetes in diabetes-prone BB Wistar rats and elevated serum and pancreas Zn concentration compared to BB rats given normal dietary Zn (Tobia *et al*. 1998). A low Zn diet (1 mg Zn/kg diet) did not alter diabetes

incidence in the BB rats at 100-days-old but pancreas and serum Zn concentrations were reduced compared to the normal Zn BB rats. High dietary Zn intakes (greater than four times recommended amounts) can impact negatively on copper (Cu)-dependent enzymes and biochemical indices of Cu deficiency in growing rats (L'Abbe and Fischer 1984a, 1984b). The Zn-supplemented BB rats had elevated serum Cu and reduced pancreas Cu concentrations (Tobia *et al.* 1998), suggesting some alteration of Cu metabolism.

Ho *et al.* (2001) demonstrated that high dietary Zn supplementation (1000 mg Zn/kg diet for 14 days prior to diabetes induction versus 50 mg Zn/kg in the control diet) reduced the effects of STZ- and alloxan-induced diabetes in mice and this was associated with elevated pancreas Zn and MT concentrations. The high Zn supplementation was short term (14 days); serum and pancreas Cu concentrations were not affected but serum Zn concentrations were doubled. Although different mechanisms may be involved in the induction of diabetes by STZ and alloxan, Zn supplementation inhibited STZ- and alloxan-induced activation of NFkB, a redox-sensitive transcription factor, and inducible nitric oxide synthase (iNOS), a downstream target of NFkB (Ho *et al.* 2001). Like the results of Apostolova *et al.* (1997), there was a dose dependent-response (and mechanism(s)) as supplementation with 500 mg Zn/kg diet provided some protection against diabetes induction, elevated pancreatic Zn *but not* MT, and partially inhibited STZ-induced NFkB activation in pancreas (Ho *et al.* 2001).

Similarly, Schott-Ohly *et al.* (2004) have reported that pre-treatment with Zn sulphate-enriched (25 mM) drinking water for 1 week before 3–5 day multiple low dose STZ injections inhibited STZ-induced up-regulation of *ex vivo* activity of NFkB and activator protein-1 (AP-1). Zn alone (i.e. no STZ injection) did not affect NFkB and AP-1, transcription factors which are required for cytokine gene activation and disease progression in chronic inflammatory diseases. Administration of Zn sulphate-enriched drinking water to parents and F1 offspring also protected female non-obese diabetic (NOD) mice from spontaneous development of Type 1 diabetes (via T cell-dependent inflammatory responses in this model) (Schott-Ohly *et al.* 2004). Less protection was achieved if only the breeding pairs or offspring were provided with Zn in the drinking water, indicating *in utero* programming of diabetes development and prevention in NOD mice. In contrast to the STZ model, Zn treatment of NOD mice induced activation of NFkB and AP-1 in islets. The authors proposed that Zn may prevent cytokine-induced apoptosis of islets in NOD mice through activation of NFkB, whereas Zn inhibition of STZ-induced NFkB activation may prevent cytokine mediated toxicity in STZ mice (Schott-Ohly *et al.* 2004). These studies illustrate the importance of understanding the mechanisms of islet cell destruction in relationship to mechanisms for Zn protection.

Zn and Type 2 diabetes models

Protective effects of Zn have also been demonstrated in rodent models of Type 2 diabetes. Very high dietary Zn supplementation (1000 mg Zn/kg diet) for 4 weeks in *ob*/*ob* mice attenuated fasting hyperglycemia and hyperinsulinemia, elevated insulin in pancreatic islets, and attenuated the abnormally high insulin secretory response to glucose in isolated pancreatic islets from *ob*/*ob* mice (Begin-Heick *et al.* 1985). Zn supplementation did not improve the response of *ob*/*ob* mice to an oral glucose tolerance test, leading the researchers to conclude that Zn supplementation did not alter the peripheral response in this model (Begin-Heick *et al.* 1985).

A lower level of dietary Zn supplementation (300 mg Zn/kg diet) for 6 weeks was effective for reducing fasting hyperglycemia and hyperinsulinemia, and reducing weight gain (i.e. better metabolic control) in young *db*/*db* mice (Simon & Taylor 2001). Unchanged liver and renal Cu concentrations and normal growth in the lean genotype suggested that the dietary Zn supplementation was not adversely affecting Cu metabolism. Conversely, a Zn-deficient diet (3 mg Zn/kg diet versus a Zn-adequate diet of 30 mg Zn/kg diet) for 6 weeks exacerbated fasting hyperglycemia in *db*/*db* mice and this was associated with reduced circulating insulin. Pancreatic Zn was reduced in *db*/*db* mice compared to the lean genotype, and Zn supplementation of *db*/*db* mice normalized pancreatic Zn to the levels of lean mice. The combination of higher pancreatic Zn

and lower circulating insulin concentrations in Zn-supplemented *db*/*db* mice versus control *db*/*db* mice suggested that Zn supplementation improved pancreatic β-cell function and/or peripheral insulin sensitivity such that less circulating insulin was required for glucose uptake. Zn has been shown to enhance tyrosine kinase phosphorylation in insulin signal transduction using *in vitro* systems (Findik & Presek 1988; Mooney & Bordwell 1992). Insulin-stimulated tyrosine kinase activity was higher in the *db*/*db* genotype, however, it was not altered in mice fed Zn-supplemented or Zn-deficient diets (Simon & Taylor 2001).

Another approach has been development of Zn-containing compounds with insulinomimetic activity. Daily intraperitoneal injections of these Zn(II) complexes for 14 days attenuated hyperglycemia, hyperinsulinemia and hyperlipidemia, and improved oral glucose tolerance and HbA1c values in KK-A^y mice (Type 2 diabetes model) without negative effects on indices of renal and hepatic function (Yoshikawa *et al.* 2001, 2002; Kojima *et al.* 2003). Similar results have been reported in GK rats (non-obese Type 2 diabetes model) given Zn(II) complexes intraperitoneally or by oral administration for 30–45 days (Fugono *et al.* 2002).

Although Zn has demonstrated protective effects in the Type 2 models of diabetes, some limitations need to be acknowledged. The genetic rodent models of Type 2 diabetes tend to have greater hyperglycaemic levels (e.g. >15 mM serum glucose) than humans (>7 mM fasting serum glucose for diagnosis of diabetes). Most of the rodent models for Type 2 diabetes are characterized by single gene mutations (e.g. mutations in leptin, leptin receptor or the agouti locus for *ob*/*ob*, *db*/*db*, and KK-A^y mice, respectively), whereas multiple gene interactions with environment are believed to contribute to Type 2 diabetes in humans. In terms of Zn, the studies have initiated Zn treatment at a relatively young age (5–9 weeks of age) for a short period of time (2–6 weeks). Elevated Zn intakes over longer periods of time could lead to toxicity. Furthermore, the safety window for high Zn intakes is relatively narrow. L'Abbe & Fischer (1984a, 1984b) have reported that four times the recommended Zn intake for rats leads to biochemical evidence of Cu deficiency. For humans, the Upper Limit (UL) for Zn intake is 40 mg/day for adults compared to the Recommended Daily Allowances of 8 and 11 mg Zn/day for females and males, respectively (Food and Nutrition Board 2001).

Further research is required to identify the mechanism(s) for the protective effects of Zn supplementation in Type 2 diabetes models, and in particular, to distinguish the effects on the pancreas versus the periphery. Studies of dietary Zn deficiency in non-diabetic rats have demonstrated that peripheral insulin resistance contributes to impaired glucose tolerance in Zn-deficient rats (reviewed by Tallman & Taylor 1999). For example, Zn-deficient rats were more resistant to exogenous insulin injections compared to pair-fed control rats (Quarterman *et al.* 1966), and had less glucose turnover during an euglycemic hyperinsulinemic glucose clamp (Faure *et al.* 1992). However, assessments of peripheral insulin resistance, insulin signalling and its downstream effects in the periphery have generally not been done in studies investigating Zn in rodent models of diabetes. Furthermore, there is evidence to support interrelationships among obesity (present in most Type 2 diabetes), leptin and Zn metabolism. For example, adipose Zn concentrations were reduced in mice with diet-induced obesity and adipose Zn was negatively correlated with serum leptin concentrations (Tallman & Taylor 2003). MT-null mice are obese and have hyperleptinemia (Beattie *et al.* 1998). Thus, peripheral effects of Zn in diabetes may extend beyond interactions with insulin and include adipocytokines and other signalling molecules.

The investigations of Zn in Type 1 diabetes models have focussed on the pancreas, however, antioxidant protection in the pancreas is also relevant in Type 2 diabetes. The pathways responsible for glucose-dependent insulin secretion appear to simultaneously increase reactive oxygen production (Fridlyand & Philipson 2004). This would suggest that increased insulin secretion in Type 2 diabetes would be accompanied by greater reactive oxygen production, and consequently more oxidative stress if the antioxidant defense system was insufficient to detoxify the increased load of free radicals. Reactive oxygen species and circulating inflammatory mediators can activate apoptotic signalling pathways in β-cells (Mandrup-Poulsen 2003). Thus, Zn may protect the pancreas in Type 2 diabetes directly as an antioxidant (Bray & Bettger 1990) or via inhibition of redox

sensitive transcription factors and signalling pathways for apoptosis and inflammatory mediators (e.g. Ho *et al*. 2001, 2004).

Conclusions

In summary, Zn supplementation is effective for preventing or ameliorating diabetes in several rodent models of Type 1 and Type 2 diabetes. Studies with chemically-induced diabetes (i.e. STZ and alloxan) indicate that the protective effects of Zn involve antioxidant mechanisms whether it is Zn alone (as an antioxidant), Zn induction of MT or Zn inhibition of redox-sensitive transcription factors (e.g. NFkB activation). Further studies are needed to identify the mechanism(s) for Zn protection in Type 2 diabetes, including pancreatic and peripheral effects. It is possible that Zn protects against progressive β-cell injury in Type 2 diabetes via mechanisms involving inhibition of oxidative stress, apoptosis and inflammation. Worldwide incidence of diabetes is increasing at an alarming rate, with Type 2 diabetes representing > 90% of cases. Given that Type 1 and Type 2 diabetes are characterized by β-cell destruction and β-cell exhaustion, respectively, there is considerable opportunity to develop preventative and therapeutic strategies for protecting pancreatic function. Experimental manipulations of Zn status in rodent models of diabetes provide a valuable approach to explore mechanisms for the protective effects of Zn; however, long term clinical studies establishing safety (lack of toxicity) and efficacy are required before any recommendations can be made for people with diabetes.

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