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Zinc transporter gene expression and glycemic control in post-menopausal women with Type 2 diabetes mellitus

Meika Foster[a], Anna Chu[a], Peter Petocz[b], Samir Samman[a,*]

[a] Discipline of Nutrition & Metabolism, School of Molecular Bioscience, University of Sydney, NSW 2006, Australia
[b] Department of Statistics, Macquarie University, NSW 2109, Australia

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ABSTRACT

Type 2 diabetes mellitus (DM) is associated often with underlying zinc deficiency and nutritional supplements such as zinc may be of therapeutic benefit in the disease. In a randomized, double-blind, placebo-controlled, 12-week trial in postmenopausal women ($n=48$) with Type 2 DM we investigated the effects of supplementation with zinc (40 mg/d) and flaxseed oil (FSO; 2 g/d) on the gene expression of zinc transporters (*ZnT1*, *ZnT5*, *ZnT6*, *ZnT7*, *ZnT8*, *Zip1*, *Zip3*, *Zip7*, and *Zip10*) and metallothionein (*MT-1A*, and *MT-2A*), and markers of glycemic control (glucose, insulin, glycosylated hemoglobin [HbA1c]). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. No significant effects of zinc or FSO supplementation were observed on glycemic marker concentrations, HOMA-IR or fold change over 12 weeks in zinc transporter and metallothionein gene expression. In multivariate analysis, the change over 12 weeks in serum glucose concentrations ($P=0.001$) and HOMA-IR ($P=0.001$) predicted the fold change in *Zip10*. In secondary analysis, marginal statistical significance was observed with the change in both serum glucose concentrations ($P=0.003$) and HOMA-IR ($P=0.007$) being predictive of the fold change in *ZnT6*. *ZnT8* mRNA expression was variable; HbA1c levels were higher ($P=0.006$) in participants who exhibited *ZnT8* expression compared to those who did not. The significant predictive relationships between *Zip10*, *ZnT6*, serum glucose and HOMA-IR are preliminary, as is the relationship between HbA1c and *ZnT8*; nevertheless the observations support an association between Type 2 DM and zinc homeostasis that requires further exploration.

Introduction

Zinc dyshomeostasis and increased levels of oxidative stress play major roles in the pathogenesis of Type 2 diabetes mellitus (DM) [1]. Zinc is known to elicit insulin-like effects and it is this property that is most likely affected by the alterations in zinc distribution and metabolism associated with DM. Zinc deficiency associated with DM may impair the ability of zinc to induce the expression of zinc binding proteins, namely metallothionein (MT), and thereby increase the risk of oxidative stress-induced damage. Other critical pathways that are affected in DM and which are influenced by zinc include those of lipid metabolism, inflammation and insulin signalling [1]. Under normal conditions zinc is found throughout the pancreas, where it forms an integral component of the insulin crystalline structure [2], serving to stabilize the insulin granule by rendering it less soluble [3]. Zinc transporter 8 (ZnT8) is responsible for delivering zinc ions from the cytoplasm of pancreatic β-cells to insulin granules, which are subsequently secreted as a zinc-rich complex. Recent evidence suggests that single nucleotide polymorphisms in the *ZnT8* gene are associated with impaired proinsulin conversion [4] and an increased risk of developing Type 2 DM [5].

Perturbed zinc homeostasis in DM appears to lead to a state of conditioned (secondary) zinc deficiency, evidenced in part by hyperzincuria that is reported to be present in the disease, and therefore zinc replenishment may exert a favourable effect. In a meta-analysis of randomised controlled trials that was conducted to determine the effect of zinc on markers of glycemic control, we reported a reduction in fasting serum glucose concentrations and a tendency for glycosylated hemoglobin (HbA1c) to decrease after zinc supplementation [6]. The interaction between glycemic markers and the gene expression of zinc transporters in humans is unclear. The aim of the present study is to examine the relationships between markers of glycemic control and the gene expression of MT and specific zinc transporters in women with Type 2 DM.

* Corresponding author at: Department of Human Nutrition, University of Otago, PO Box 56, Dunedin 9054, New Zealand.
E-mail address: samir.samman@otago.ac.nz (S. Samman).

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Zinc transporters were selected to canvass import and export functions across both the plasma and intracellular vesicular membranes and/or because they had been shown to be zinc-responsive in previous studies in humans, animal models and cell culture systems [7].

Methods and materials

The trial design and participant details have been reported previously in papers exploring the effects of zinc and flaxseed oil (FSO) supplementation on markers of lipidemia and glycemia [8] and the relationships between zinc transporter and MT gene expression and inflammation [9]. In the present study we undertook a secondary analysis to determine the relationship between markers of glycemia and the gene expression of zinc transporters. Briefly, women were enrolled in the trial if they were post-menopausal (no menses for >12 mo) with Type 2 DM, and had a glomerular filtration rate and micro-albumin/creatinine ratio in the normal range. Exclusion criteria were: the use of insulin; smoking; and diagnosis with any current major illness other than DM. The Human Research Ethics Committee of the University of Sydney approved the study protocol and all participants provided written informed consent. The protocol was registered at http://www.ClinicalTrials.gov, with the identifier NCT01505803.

The study was a randomized, double-blind, placebo-controlled trial conducted over 12 weeks. Upon enrolment, 48 participants were randomized into four equal groups to receive 40 mg/d elemental zinc, 2 g/d FSO, both zinc and FSO, or placebo. Venous blood samples were collected at 4-weekly intervals from participants after an overnight fast of at least 10 h. Cell preparation tubes (CPT vacutainers; Becton Dickinson) were used for the isolation of peripheral blood mononuclear cells (PBMC), serum gel tubes for glucose and insulin analyses, EDTA tubes for analysis of HbA1c, and trace metal tubes (Becton Dickinson) for plasma zinc analysis. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR) calculation [10].

Zinc transporter and metallothionein gene expression

The method for measuring zinc transporter and MT mRNA expression has been described previously [9,11]. In brief, PBMC from individual samples were extracted and total RNA was prepared (Applied Biosystems-Life Technologies Australia Pty Ltd, Victoria, Australia) and reverse transcribed into cDNA (Invitrogen-Life Technologies Australia Pty Ltd, Victoria, Australia) using commercially available kits. RNA integrity was assessed. Purity and yield of the isolated RNA were determined by UV spectrophotometry, with all samples generating an A260:A280 ratio within the range of 1.8–2.1. RNA integrity was verified by ethidium bromide staining after 1% denaturing agarose gel electrophoresis.

Relative quantification of zinc transporter mRNA (*ZnT1*, *ZnT5*, *ZnT6*, *ZnT7*, *ZnT8*, *Zip1*, *Zip3*, *Zip7*, and *Zip10*), and MT (*MT-1A*, and *MT-2A*) was conducted using Taqman real-time PCR (ABI 7500 Fast Sequence Detection System; Applied Biosystems-Life Technologies Australia Pty Ltd, Victoria, Australia). Expression levels were normalized to 18S rRNA and quantified using the ΔC_P method, and fold change was quantified using the $\Delta\Delta C_P$ method.

Statistical analysis

Differences in the concentrations of biochemical measures over time within each treatment group were assessed using multivariate analysis. Post-hoc investigations using student *t*-tests were undertaken to compare the results of all participants who received zinc supplements with all participants who were not supplemented with zinc. Comparisons between participants who expressed *ZnT8* mRNA and participants in whom *ZnT8* mRNA was not expressed were conducted using unpaired student *t*-tests. Principal component analysis was used to identify "clusters" of zinc transporter and MT gene expression, and multivariate and univariate ANOVA models were used to determine whether the glycemic markers (serum glucose, serum insulin, HOMA-IR, HbA1c) predicted any of the specific zinc transporter or MT mRNA or any of the identified clusters. Statistical analyses were carried out using SPSS v18. $P < 0.01$ was interpreted as statistically significant, with the exception that a conservative value of $P < 0.001$ was adopted for multivariate and univariate analyses due to the large number of test results under investigation.

Table 1
Biomarkers of glycemic control at baseline and week 12 in participants who were supplemented with zinc ($n = 23$) or not supplemented with zinc ($n = 20$).

	Baseline	Week 12	Change
Glucose (mmol/L)			
All	6.94 ± 0.26		
Zn	6.77 ± 0.17	6.87 ± 0.25	0.11 ± 0.17
Control	7.15 ± 0.53	7.00 ± 0.66	−0.16 ± 0.22
HbA1c (%)			
All	6.68 ± 0.15		
Zn	6.55 ± 0.09	6.73 ± 0.12	0.18 ± 0.09
Control	6.83 ± 0.30	6.83 ± 0.32	−0.01 ± 0.07
Insulin (pmol/L)			
All	68.5 ± 5.51		
Zn	63.0 ± 8.18	69.8 ± 10.20	6.78 ± 5.32
Control	74.8 ± 7.18	68.9 ± 4.95	−5.9 ± 5.22
HOMA-IR			
All	3.52 ± 0.32		
Zn	3.17 ± 0.43	3.54 ± 0.49	0.36 ± 0.28
Control	3.93 ± 0.47	3.53 ± 0.37	−0.40 ± 0.33

Data expressed as mean ± SE. HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model of assessment–insulin resistance.
Reference ranges for outcome measures are: glucose, 3.0–7.7 mmol/L; insulin, 10–96 pmol/L; HbA1c, 3.5–6.0%.

Results

Forty-three participants completed the trial and were included in the statistical analysis. The mean age and BMI were, respectively, 65.0 ± 7.8 y (mean ± SD) and 28.6 ± 5.1 kg/m^2. The plasma zinc concentration of participants at baseline was 12.8 ± 0.3 μmol/L (mean ± SE) and within the reference range (10–18 μmol/L). Serum concentrations of the glycemic measures were within their respective reference intervals, except for HbA1c, which was above the normal range (Table 1).

Significant increases in plasma zinc were observed over time in the groups supplemented with zinc and both zinc and FSO ($P = 0.002$ and $P = 0.019$, respectively), but not in the groups supplemented with FSO alone or placebo. There were no statistically significant effects of zinc supplementation on HbA1c, serum glucose and insulin concentrations, or HOMA-IR when analysed by treatment group [8]; nor when grouped according to whether participants received ($n = 23$) or did not receive ($n = 20$) zinc as part of their intervention (Table 1).

There were no significant differences in the baseline expression of any of the zinc transporter or MT mRNA in participants who received zinc compared to those who did not receive zinc as part of their intervention [9]. *ZnT8* mRNA expression was detected only in 21 participants; compared to participants in whom *ZnT8* mRNA was not expressed, participants who expressed *ZnT8* mRNA were observed to have higher HbA1c levels (6.75% vs. 6.31%; $P = 0.006$).

There were no significant differences between baseline and week 12 zinc transporter (including *ZnT6* and *Zip10*; Fig. 1), and MT mRNA expression in participants, irrespective of zinc