

**INVESTIGATIONS INTO THE ROLE OF
ZINC AND ZINC TRANSPORTERS IN THE
PATHOGENESIS OF TYPE 2 DIABETES IN
db/db MICE**

By

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Abstract

Zn is critical for the synthesis, storage and release of insulin and abnormalities in Zn and Zn transporters occur in type 1 and 2 diabetes. However, the mechanism by which Zn is regulated in islet cells is still poorly understood. The major goal of this thesis was to investigate the role of Zn and Zn transporters in the pathogenesis of normal and type two diabetic pancreatic islets, using the type 2 db/db mouse model. There is limited information available on the physiological role of Zn and Zn transporters in these mice. The following hypotheses were tested. 1) There is an early loss of Zn in the development of type 2 diabetes which contributes to the transition to established diabetes; 2) The loss of Zn causes a block in insulin maturation resulting in impaired glucose responsiveness, hyperglycemia and decline in beta cell function; 3) This loss of Zn is due to alterations in Zn transporter proteins and metallothionein at the gene and protein level. Specifically changes in the organelle Zn transporters ZnT7 and ZnT8 result in the block in insulin maturation, while dysregulation of the inflammation related plasma membrane Zn transporter protein ZIP14 contributes to inflammation that results in further beta cell dysfunction. The major aims of the project were to determine whether in early and late diabetes there are changes in 1) total and labile Zn and metallothionein, 2) Zn transporter gene expression; and 3) Zn transporter proteins. Whole pancreata from the db/db mice and age matched controls at various ages were used to investigate Zn, metallothionein protein, gene expression and subcellular distribution of Zn transporters and Zn related proteins. Immunofluorescence, immunoperoxidase and western blotting were used to investigate the Zn transporter protein expression and distribution. The major findings in this study were in early diabetes 1) loss of Zn occurred in the labile islet beta cells Zn pools without decrease in systemic Zn ; 2) There were no changes at the gene level of Zn transporters ZnT1-10 and ZIP1-14 or metallothionein; 3) There was a significant increase in islet ZnT7 protein with a golgi like appearance; 4) ZnT8 protein was downregulated in islet beta cells but not alpha cells; 5) ZIP4 was expressed almost exclusively in the somatostatin producing delta cells; 6) ZIP14 staining was significantly increased and coincided with islet macrophages. Changes in ZnT7, ZnT8 and ZIP14 expression may be factors leading to the loss of islet beta granule Zn. ZIP4 may be the major influx transporter for Zn in delta cells, ZnT8 is the transporter regulating Zn in insulin secretory granules and ZIP14 may be a novel marker of macrophage infiltration in diabetic islets. There are

two potential clinical implications. The first is in understanding better the early events in development of type 2 diabetes, how these are influenced by Zn status and whether Zn supplements have a role to play in slowing down the transition from pre-diabetes to established diabetes. The second is a better understanding of islet Zn homeostasis with potential benefits for outcomes of islet transplantation.

It is the branch that bears the fruit,
That feels the knife,
To prune it for larger growth,
A fuller life
Though every budding twig be loped
And every grace
Of swaying tendril, springing leaf,
Be lost a space
O thou whose life of joy seems left,
Of beauty shorn;
Whose aspirations lie in dust,
All bruised and torn
Rejoice tho each desire, each dream
Each hope of thine
Shall fall and fade it is the land
Of love divine
That holds the knife that cuts and breaks
With tenderest touch,
That thou, whose life has borne,
Some fruit
Mayst now bear much

Annie Johnson Flint

Declaration

I, Mariea Dencey Bosco certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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.....

Mariea Dencey Bosco

Date:

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“Obstacles don’t have to stop you. If you run into a wall, don’t turn around and give up. Figure out how to climb it, go through it, or work around it.”

- Michael Jordan

“Success is not the key to happiness. Happiness is the key to success. If you love what you are doing, you will be successful.”

- Herman Cain

Abbreviations used in this thesis

AAS	Atomic Absorption Spectrometry
Ca ²⁺	Calcium
DNA	Deoxyribonucleic acid
ER	Endoplasmic Reticulum
FBS	Foetal bovine serum
FITC	Fluorescein isothiocyanate
GK	Goto Kakizaki rats
GLMS	Gaussian generalised linear models
GLP-1	Glucagon like peptide 1
GLUT2	Glucose transporter 2
IL-1 β	Interleukin 1 beta
IL-6	Interleukin 6
MIN6	Mouse Insulinoma cell line
mM	milli Molar
MRE	Metal response element
mRNA	Messenger RNA
MT	Metallothionein
MTF-1	Metal transcription factor 1
nM	nano Molar
Ob	Obese gene
°C	Degrees Celsius
PBS	Phosphate buffered saline
PKC	Protein kinase C
pM	pico Molar
RER	Rough endoplasmic reticulum
RIN	Rat Insulinoma Cell line
RT	Reverse transcriptase
SEM	Standard error of mean
SNP	Single nucleotide polymorphism
TLDA	Taqman low density array cards
TNF- α	Tumour necrosis factor alpha
TPEN,	N,N',N'-tetrakis-(2-pyridyl-methyl) ethylenediamine
TS-Q	6-Methoxy-(8-p-toluenesulfonamido)quinoline
UV	Ultraviolet
ZINPYR-1	4',5'-Bis[bis(2-pyridylmethyl)aminomethyl]-2',7'-dichlorofluorescein
ZIP14	Zinc transporter SLC39A14
ZIP4	Zinc transporter SLC39A4

ZIP5	Zinc transporter SLC39A5
Zn	Zinc
ZnT7	Zinc transporter SLC30A7
ZnT8	Zinc transporter SLC30A8
μL	micro Liter
μM	micro Molar

Publications and Awards Arising from Thesis

Peer-Reviewed Publications

Review: Rev Diabet Stud. 2010 Winter;7(4):263-74. doi: 10.1900/RDS.2010.7.263.
Epub 2011 Feb 10. Zinc and zinc transporter regulation in pancreatic islets and the potential role of zinc in islet transplantation. Bosco MD, Mohanasundaram DM, Drogemuller CJ, Lang CJ, Zalewski PD, Coates PT.

Zinc and Zinc Transporters in Macrophages and Their Roles in Efferocytosis in COPD. Hamon R, Homan CC, Tran HB, Mukaro VR, Lester SE, Roscioli E, Bosco MD, Murgia CM, Ackland ML, Jersmann HP, Lang C, Zalewski PD, Hodge SJ. PLoS One. 2014 Oct 28;9(10):e110056. doi: 10.1371/journal.pone.0110056. eCollection 2014.

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Post translational change in the regulation of Zinc and Zinc transporters in a Type 2 Diabetic db/db mice pancreatic islets.

Mariea D Bosco, Daisy M Mohandasundram, Chris Drogemullar, Peter Zalewski, Toby P Coates

Differential Regulation of Zinc Transporters and Metal Binding proteins in human Type 1 and Type 2 Diabetes.

Daisy Mohanasundaram, Mariea Bosco, Peter Zalewski, Chris Drogemullar, Tom Loudovaris, Tom Kay, Toby Coates

TSANZ ANNUAL SCIENTIFIC MEETING 2011: Mini Oral

Down regulation of Zinc transporter ZIP14 and ZnT8 in native pancreatic islets of type 2 diabetic db/db mice

Bosco Mariea Dencey, Zalewski Peter, Mohanasundram Daisy, Coates PTH.

Alteration of Zinc and Differential expression of Zinc transporters in Diabetic pancreatic islets.

Mohanasundram Daisy, Drogemullar Chris, Bosco Mariea, Zalewski Peter, Coates Toby.

ASMR Adelaide: Poster presentation

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Alteration in the regulation of zinc and zinc transporters can contribute to beta cell dysfunction in type 2 diabetic pancreatic islets

Mohanasundram Daisy, Drogemullar Chris, Bosco Mariea, Zalewski Peter, Coates Toby.

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Bosco MD, Mohanasundram D, Lester S, Zalewski P, Mee C, Drogemuller C, Miliner C, Coates P.T.H.

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Meeting- Adelaide-Australia: Poster presentation

Down regulation of zinc transporter 8 in type 1 diabetic pancreatic islet

Mohanasundram Daisy, Drogemullar Chris, Bosco Mariea, Zalewski Peter, Coates Toby.

Association of type 2 diabetes with significant reduction of zinc level in db/db mice liver

M.D. Bosco, P. Zalewski, D. Mohanasandrum, P.T.H. Coates.

International Pancreas Islet Transplant Association 2011: Poster Presentation

Role of zinc and zinc transporters in diabetic pancreatic islets

D. Mohanasundaram, C. Drogemullar, M. Bosco, C. Lang, P. Zalewski, T. Coates

Zn in Biology International Conference 2012: Melbourne, poster

Age related reduction in zinc concentration in type 2 diabetic db/db murine livers

Bosco, D Mohanasundaram, P Zalewski, C Cowley, A Rofe, PTH Coates.

Zinc transporter analysis in human type 1 and type 2 diabetic pancreatic islets

D. Mohanasundaram, C. Drogemuller, T.Loudovaris, L.Mariana, M.Bosco, C. Lang, P.

Zalweski, T. Coates

ASMR 2012 Adelaide: Oral presentation

Regulation of Metallothionein and zinc in liver and pancreas in type 2 diabetic db/db mice

Mariea Bosco, Daisy Mohanasundram, Peter Zalewski, Patrick Toby H Coates

TSANZ Annual Scientific Meeting 2012: Canberra, Mini Oral

Zinc regulation in liver and pancreas in type 2 diabetic db/db mice

Bosco Mariea, Mohanasundram Daisy, Zalewski Peter, Drogemuller, Coyle Peter, ROFE Allan, Coates Toby.

Australian Diabetes Society 2012: Gold Coast, Poster Presentation

Leptin deficient type 2 diabetic db/db mice have altered zinc and zinc transporter metabolism.

Mariea D Bosco, Daisy M Mohandasundram, Chris Drogumullar, Peter Zalewski, Carina Cowley, Peter Coyle, Allan M Rofe, Toby P Coates

Research Day, Basil Hetzel Institute 2012: Adelaide, Poster presentation (mini oral)

Zinc and Zinc transporter ZnT8 regulation altered in prediabetic db/db mice

Bosco Md, Mohanasundram D, Zalewski P, Rofe A, Coyle P, Drogemuller C, Coates PTH.

The Australian Health and Medical Research Congress 2012: Oral /Poster presentation

Alteration of zinc and zinc transporter metabolism in a type diabetic db/db mice model

Mariea D Bosco, Daisy M Mohandasundram, Chris Drogumullar, Peter Zalewski, Carina Cowley, Peter Coyle, Allan M Rofe, Toby P Coates

TSANZ 2013: Oral Presentation

Loss of Zinc and alteration of Zinc transporters in db/db mice pancreatic islets in early type 2 diabetes.

Bosco Mariea, Mohanasundram Daisy, Zalewski Peter, Drogemuller C, Coyle Peter, Rofe Allan, Coates PTH.

ASMR 2013: Oral Presentation

Early changes in zinc and zinc transporters in a type 2 diabetic db/db mice pancreatic islets.

Mariea Bosco, Daisy Mohansaundram, Chris Drogemuller, Peter Zalewski, Peter Coyle, Allan Rofe, Patrick Toby H Coates.

Australain Diabetes Society :Poster

Inflammation induced ZIP14 transporter upregulated in pancreatic islets of type 2 diabetic db/db mice

Mariea Bosco, Daisy Mohanasundaram Chris Drogemuller, Peter Zalewski, Peter Coyle, Allan Rofe, Patrick Toby H Coates

Australian Diabetes Islet Study Group: Poster

Islet specific reduction of zinc and alteration of zinc transporters in pancreatic islets of a type 2 diabetic db/db mice model

Bosco Mariea, Mohanasundram Daisy, Zalewski Peter, Drogemuller C, Coyle Peter, Rofe Allan, Coates PTH.

International Zinc Biology Conference 2014 Asilomar Camp Grounds California

Role of Zn and Zn transporters in the pathogenesis of type 2 diabetes in db/db mice

Mariea D. Bosco, Daisy Mohanasundaram, Claire F. Jessup, Claudine S. Bonder, Darling Rojas-Canales, Chris Drogemuller, Tom Loudovaris, Tom W.H. Kay, Susan E. Lester, Shane Grey, Peter D. Zalewski and Patrick. T Coates

TSANZ and Amgen 2013 Young Investigator Book Prize \$500, 2013
IPITA-TTS Young Investigator Travel 2013 for best Abstract \$1100, 2013
Discipline of Medicine Travel Grant \$800, 2012
TSANZ Travel grant \$2500, 2013
Robinson Institute Travel Grant \$1800, 2013
Walter and Duncan Trust Travel Grant \$1500, 2013
Freemasons Trevor Prescott Scholarship \$5000, 2014
International Zinc Biology Conference Book prize and Poster award