ETHANOL-RELATED TERATOGENICITY AND NEUROBEHAVIOURAL IMPAIRMENTS: INFLUENCE OF DIETARY ZINC SUPPLEMENTATION DURING PREGNANCY

A thesis submitted for the degree of Doctor of Philosophy

by

BROOKE LEE SUMMERS
B.Sc. (Biomedical Science) (Honours)

Division of Clinical Biochemistry,
Institute of Medical and Veterinary Science/ Hanson Institute, Adelaide, South Australia

and

The Department of Physiology,
The University of Adelaide, Adelaide, South Australia

October 2008

TABLE OF CONTENTS

ABBREVIATIONS

°C	Degrees Celsius
µg	microgram
µl	microlitre
µmol	micromole
AE	Acrodermatitis Enteropathica
Ag	Silver
ARBD	Alcohol Related Birth Defects
ARND	Alcohol Related Neurdevelopmental Disorder
Au	Gold
BAC	Blood Alcohol Concentration
Bi	Bismuth
Cd	Cadmium
cm	centimetre
CNS	Central Nervous System
CRL	Crown Rump Length
Cu	Copper
d	day
dL	decilitre
DNA	Deoxyribonucleic Acid
EDC	Ethanol Derived Calories
EP	Escape Platform
FAS	Fetal Alcohol Syndrome

FASD	Fetal Alcohol Spectrum Disorder
g	gram
GD	Gestational Day
GLM	General Linear Model
GRE	Glucocorticoid Response Element
h	hour
HCl	Hydrochloric acid
Hg	Mercury
hZTL1	human ZnT-like transporter 1
IMVS	Institute of Medical and Vetinary Science
kg	kilogram
L	Litre
LPS	Lipopolysaccharide
LSD	Least Significant Difference
mg	milligram
Mg	Magnesium
min	minute
ml	millilitre
mm	millimetre
MRE	Metal Response Element
MT	Metallothionein
MTF	Metal Transcription Factor
n	number

NaCl	Sodium Chloride
NAD	Nicotinamide Adenine Dinucleotide
NTD	Neural Tube Defect
ORT	Object Recognition Task
PD	Postnatal Day
ppm	parts per million
REML	Restricted Maximal Likelihood
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
s	seconds
SEM	Standard Error of Mean
TF IIIA	Transcription Factor III A
TNF-α	Tumor Necrosis Factor alpha
v	volume
VSD	Ventricular Septal Defect
w	weight
Zn	Zinc
$ZnSO_4$	Zinc Sulphate

ABSTRACT

Ethanol consumption during pregnancy can result in wide range of negative outcomes, including pre- and post-natal mortality, growth retardation, physical abnormalities and brain deficits, manifested as behavioural impairments. These outcomes can result from "binge-drinking" (generally defined as >5 standard drinks on a single occasion) or chronic ethanol consumption. Ethanol-induced zinc (Zn) deficiency is one of the mechanisms proposed as a cause of ethanol teratogenicity. We have previously demonstrated in mice that ethanol exposure on gestational day (GD)8 (during organogenesis) can alter Zn homeostasis by inducing the Zn-binding protein metallothionein (MT) in the maternal liver. This causes plasma Zn concentrations to decrease as Zn redistributes into the liver, and consequently decreases the fetal Zn supply and increases the risk of teratogenicity. Subcutaneous Zn treatment with ethanol on GD8 can prevent the deleterious effects of ethanol on the fetus (i.e. physical abnormalities and spatial memory impairments). The main objective of this thesis was to investigate whether a less invasive approach of giving dietary Zn supplementation throughout pregnancy could provide similar protective benefits against a range of adverse outcomes caused by prenatal binge or chronic ethanol exposure.

Binge ethanol exposure in early pregnancy (i.e. where mice are injected with 25% ethanol (0.015 ml/g) intraperitoneally at 0 and 4 hours on GD8) significantly increased the incidence of birth abnormalities measured on GD18. These included craniofacial abnormalities (microphthalmia, anophthalmia) and limb defects. Ethanol

also increased postnatal mortality between birth and postnatal day (PD)60. In a separate study, offspring from dams given ethanol on GD8 were subjected to a physical and behavioural screening protocol (including tests for vision, olfactory, exploratory, anxiety and motor impairments) and subsequently a cohort of phenotypically-normal offspring were randomly selected for testing in a cross-maze escape task (for spatial learning and memory) and an object recognition test (for short-term non-spatial memory). While ethanol did not affect behaviour measured during screening, it resulted in spatial memory and object recognition memory impairments in adult offspring. The most important finding was that dietary Zn supplementation throughout pregnancy significantly increased plasma Zn concentrations at the time of ethanol exposure (avoiding the "typical" ethanol-induced decrease in plasma Zn) and prevented all negative outcomes resulting from early ethanol exposure (birth abnormalities, mortality, spatial and object recognition memory impairments). In the chronic ethanol mouse model (i.e. where mice were fed a liquid diet containing 27 % v/v ethanol-derived calories from GD6-18), ethanol did not affect offspring growth between birth and PD21 or spatial memory in adult offspring, thus, the influence of Zn supplementation could not be examined for these parameters. While ethanol decreased offspring weight at PD50 and increased mortality between birth and PD40, they were not prevented by Zn supplementation throughout pregnancy.

The findings from this thesis emphasise that organogenesis is a particularly vulnerable period to ethanol exposure and even a binge of ethanol during this time

can result in dysmorphology, mortality and spatial and object memory impairments in adulthood. In addition, dietary Zn supplementation is protective against the deleterious effects of binge ethanol exposure in early pregnancy.

DECLARATION

This thesis 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.

I give consent for this copy of my thesis being made available in the University Library.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works.

- **Summers BL,** Henry C, Rofe AM, Coyle (2008) Dietary Zn supplementation throughout pregnancy prevents spatial and object recognition memory impairments caused by early prenatal ethanol exposure. Behavioural Brain Research, 186, pg 230-238.

Brooke Lee Summers

Signature…… Date……..

ACKNOWLEDGMENTS

I would like to thank my supervisors Dr Peter Coyle and Dr Allan Rofe, for their guidance and support over the duration of my PhD. In particular Peter who always made himself available for when I needed assistance (which was quite often at certain times of our study!!) and I certainly could not have asked for better supervisors.

Thanks also to a number of people I have had associations with in the Department of Clinical Biochemistry and the Institute of Medical and Veterinary Science. In particular, members of our metals research group, Joanne Chua, Jenny Fung, Nancy Tran, Matthew Rees, as well as Lisa LaVence and Catherine Henry (for their technical assistance with our behavioural studies), Michael Haywood (for passing on his expertise on atomic absorptions spectroscopy for tissue and diet Zn analysis) and Paul Anderson (for his general guidance during my PhD). A special mention must also go to Kate Dowling (Biometrics SA), Thomas Sullivan and Sue Lester, who helped with analysing our results at various stages during the study.

Thankyou to the foundations that financially supported the research undertaken in this thesis; The Channel 7 Children‟s Research Foundation (Binge drinking studies) and The Australian Brewers Association (Chronic alcohol study).

I would like to thank my parents, grandma and sister for their support throughout my study (in particular grandma who prayed so hard for me to hurry up and complete my

thesis that her rosary beads wore down and broke!). It took a little longer than expected to complete my PhD but I appreciated all the support throughout my student life to help me get through all the difficult times.

I would also like to thank my husband, Paul, for his love and support over the past 4 years and for providing me with a well deserved break from thesis-writing (our wedding day and honeymoon). Being married to a PhD student is not always fun when there is research and writing to be done, but you always helped and supported me during the stressful times and made things as easy as possible for me to get my work done. FINALLY!!!

PUBLICATIONS ARISING FROM THIS THESIS

Summers BL, Henry C, Rofe AM, Coyle P (2008) Dietary zinc supplementation throughout pregnancy prevents spatial and object recognition memory impairments caused by early prenatal ethanol exposure. Behavioural Brain Research, 186, 230-238. (Research from Chapter 3 of this thesis)

Summers BL, Rofe AM, Coyle P (2008) Dietary zinc supplementation throughout pregnancy protects against fetal dysmorphology and improves postnatal survival after prenatal ethanol exposure in mice, submitted in Alcoholism: Clinical and Experimental Research, [ACER-D-08-1929R2] (Research from Chapter 2 of this thesis)

PUBLICATIONS FROM ASSOCIATED RESEARCH

Summers BL, Rofe AM, Coyle P (2006). Prenatal zinc treatment prevents spatial learning and memory impairments caused by ethanol exposure in mice. Pediatric Research, 59 (1), 6-70.

Coyle P, Tran N, Fung JN, **Summers BL**, Rofe AM (2008) Maternal dietary zinc supplementation prevents aberrant behaviour in an object recognition task in mice offspring exposed to LPS in early pregnancy. Behavioural Brain Research, Aug 28 [Epub ahead of print]

Coyle P, Carey L, Martin S, **Summers BL**, Rofe A (2008) Ethanol mediated dysmorphology and its relationship to the ontogeny of maternal liver metallothionein. Submitted in Alcoholism: Clinical and Experimental Research, [ACER-D-08-2223].