AN INVESTIGATION INTO THE EFFECT OF GLUCOSAMINE ON REPRODUCTIVE OUTCOMES IN THE MOUSE

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May 2012

A thesis submitted to the University of Adelaide to fulfill the requirements of the degree of Doctor of Philosophy

Contents

Abstract

It is well established that conditions experienced in utero by the developing fetus can elicit permanent effects on the post natal period. Although not as well understood, a growing body of research also suggests that this can also occur in response to peri-conceptional insult. Glucosamine (GlcN) is a popular dietary supplement that is also used experimentally as a hyperglycaemic mimetic. The work contained in this thesis tests the hypothesis that pre and peri-conceptional exposure to GlcN has adverse effects on reproductive outcomes in the mouse.

Preliminary experiments (Chapter 2) confirmed that the inclusion of GlcN into the in vitro maturation (IVM) media used for mouse cumulus oocyte complex (COC) maturation, reduced oocyte developmental potential. Subsequent experiments (Chapter 3) demonstrated that the inhibition of O-linked glycosylation of unknown proteins reversed the effects of GlcN. It was also shown that GlcN exposure during IVM altered Pentose phosphate pathway (PPP) activity within the oocyte.

As predicted, preliminary in vivo experiments performed in Chapter 4 showed that maternal, peri-conceptional GlcN administration compromised fetal development. This was seen by a decreased mean implantation rate and litter size as well as an increase in the proportion of fetal resorptions on gestational day 18 (d18), and provided the impetus to examine the in vivo effects of GlcN exposure more carefully.

It was subsequently hypothesized that these adverse effects would be heightened if given to mice with overweight-induced metabolic pathologies. In contrast to Chapter 4 outcomes, GlcN elicited no effects on d18 implantation, resorption or litter size parameters, but did reduce fetal weight. Furthermore, birth defects were higher in mice given GlcN and maintained on a low fat (LF) diet. An additional cohort of mice was allowed to give birth, and offspring were assessed for 16 weeks. There was an unexpectedly high death rate in the offspring of mice maintained on a high fat (HF) diet but not given GlcN, therefore preventing optimally controlled post natal analyses to occur. Of the remaining mice, a number of physiological differences were detected within GlcN-exposed groups.

Since the principle difference between mice in Chapters 4 and 5 was maternal age, an addition experiment investigating the effects of peri-conceptional GlcN exposure in 8 week and 16 week old mice was undertaken (Chapter 6). Consistent with previous results, GlcN treatment reduced mean implantation rate and litter size only in 8 week old mice and reduced fetal weigh and length solely in 16 week old mice. Increased birth defects were also detected in the HF group given GlcN.

Collectively these results provide important insights into the importance of optimal conditions during the peri-conceptional period to facilitate successful subsequent development. They also provide evidence that GlcN is a simple but effective tool that can be used to further elucidate the impact of hyperglycaemic exposure during the early developmental period. This is of key importance given the escalating instances of diabetes and obesity in current day Western society, and the associated complications that these conditions elicit on reproductive parameters.

Declaration

This work contains no material which has been accepted for publication for the award of any other degree or diploma in any other University or other tertiary institution to Cheryl Schelbach, 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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- Schelbach, C. J., Robker, R. L., Bennett, B. D., Gauld, A. D., Thompson, J. G. & Kind, K. L. (2012). "Altered pregnancy outcomes in mice following treatment with the hyperglycaemic mimetic, glucosamine, during the periconception period." Reproduction, Fertility & Development *(in press).*

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Publications arising from this thesis

Papers

Paper arising from work presented in chapters 2 and 3

Schelbach, C.J., Kind, K.L., Lane, M. and Thompson, J.G. (2009) "Mechanisms contributing to the reduced developmental competence of glucosamine exposed mouse oocytes." Reproduction, Fertility and Development**.** **22**(5): 771–779.

Paper arising from work presented in chapters 4 - 6

Schelbach, C. J., Robker, R. L., Bennett, B. D., Gauld, A. D., Thompson, J. G. & Kind, K. L. (2012). "Altered pregnancy outcomes in mice following treatment with the hyperglycaemic mimetic, glucosamine, during the periconception period." Reproduction, Fertility & Development *(in press).*

Media Releases

Schelbach, C. J. and Thompson, J. G. (2007) Interview with ABC radio "The effects of glucosamine on fertility".

Schelbach, C. J., Thompson, J. G. and Stankawicz, M. (2006) Interview with Channel 10 news "The impact of glucosamine on fertility".

Conference Presentations

Schelbach, C.J., Kind, K. L. & Thompson, J. G. Perturbations in fetal development follow short-term, in vivo glucosamine administration in mice. Proceedings from the 41st Annual Meeting of the Society for the Study of Reproduction; 2008 May 27 -30; Kailua- Kona, Hawaii,

Schelbach, C, J., Kind, K.L., Lane M & Thompson, J. G.*The Perturbation in Fetal Outcomes that follows in vivo Glucosamine Exposure is Heightened by Obesity.* Proceedings from 1st The University of Adelaide 2007 Post Graduate Research Expo; 2007 ; Adelaide, South Australia.Adelaide, The University of Adelaide.

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Schelbach, C, J., Kind, K.L., Lane M & Thompson, J. G*. Abberant Murine Embryonic development following Glucosamine exposure during IVM or embryo Culture..* Proceedings from the 2005 ESA/SRB scientific meeting; 2005, August 4 - 7; Perth, Western Australia.

Schelbach, C, J., Kind, K.L., Lane M & Thompson, J. G*. Abberant Murine Embryonic development following Glucosamine exposure during IVM or embryo Culture.* Proceedings from the 2005 ASMR scientific meeting; 2005 June 2 ; Adelaide, South Australia.

Acknowledgements

I would first and fore mostly like to thank my supervisors Assoc. Prof. Jeremy Thompson and Dr Karen Kind for the opportunity to undertake this PhD. You both show such a remarkable level of tenacity and dedication to your research pursuits, and your commitment to sharing your knowledge and vision was omnipresent. In addition to the countless discussions and all of the invaluable feedback and advice that you both provide, I also owe a big thank you to Karen for her copious feedback on what seemed like at times the 'Never Ending Story' of chapter versions.

In addition, I would also like to extend my gratitude to a number of other people who provided me with assistance. Dr Michelle Lane and Dr Rebecca Robker were particularly helpful with providing technical advice and shaping the direction of the research that was undertaken. Rebecca Kelley, Ashley Gould and Brenton Bennett all provided me with an extra pair of hands when I needed it, Dave Froiland was the Confocal whiz, and Annie Whitty and Sandy Piltz worked behind the scene to help keep the lab running. Although there are too many to mention individually, I would also like to thank my fellow students for their support and advice, and of course also for all of the laughs that we had along the way.

I also need to say a special thank you to all of my family and friends for everything from recruiting everyone around you to collect the 'mouse houses', to putting up with my erratic schedule and finally in the end helping me out immensely with babysitting.

And last but not least, I also need to say a big thank you to my wonderful partner John for his patience and support. I am dedicating this thesis to you John and our daughter Genevieve. Now that these chapters are completed I'm looking forward to moving forward into the next chapter of our lives, and you can finally have your pH – free party!

I can no other answer make, but, thanks, and thanks.

William Shakespeare

Abbreviations

Abdo - abdominal

Akt - protein kinase B

ANOVA - analysis of variance

ATP - adenosine triphosphate

BADGP - benzyl-2-acetoamido-2-deoxy-α-D-galactopyranoside

BCB - brilliant cressyl blue

BMI – body mass index

BSA - bovine serum albumin

CAM - complementary and alternative medicine

cAMP - cyclic adenosine monophosphate

CC - cumulus complex

CDCFDA - 5,6-carboxyl-2',7'-dichlorodihydrofluorescein diacetate

CL - corporo lutea

COC - cumulus oocyte complex

D14.5 – day 14.5

D18 - day 18

DCDHFDA - 2',7'-dichlorodihydrofluorescein diacetate

eCG - equine chorionic gonadotrophin

EtOH - ethanol

FBS - fetal bovine serum

FFA – free fatty acids

FSH - follicle stimulating hormone

G6PDH - glucose-6-phosphate dehydrogenase

G-6-phosphate - glucose-6-phosphate

GFPT - glutamine fructose-6-phosphate transaminase

GlcN - glucosamine

GlcN-6-P - glucosamine-6-phosphate

GLUT - glucose transporter

GRP 78 - glucose-regulated protein 78

GSH - reduced glutathione

GSSG - oxidized glutathione

H_2O_2 - hydrogen peroxide

HAS - hyaluronic acid synthase

HBP - hexosamine biosynthetic pathway

hCG - human corionic gonadotrophin

HF - high fat

HF+GlcN - high fat with glucosamine

HF-GlcN - high fat without glucosamine

HSA - human serum albumin

IGF-1 - insulin like growth factor 1

IP - intraperitoneal

IRS - insulin receptor substrate

IVF - in vitro fertilisation

IVM - in vitro maturation

JNK - c-Jun N-terminal kinase

LF - low fat

LF+GlcN - low fat with glucosamine

LF-GlcN - low fat without glucosamine

MCT proteins - H^+-monocarboxylate cotransporter proteins

mRNA - messenger ribonucleic acid

NADH - nicotinamide adenosine dinucleotide

NADPH - nicotinamide adenine dinucleotide phosphate

NADPH - nicotinamide adenine dinucleotide phosphate

NEFA - non-esterified fatty acids

O-GlcNAcase - β-N-acetylglucosaminidase

OGT - β-linked-O-GlcNAc transferase

PA – palmitic acid

PAI - plasminogen activator inhibitor

PBS - phosphate buffered solution

PEP - phosphoenolpyruvate

PI3-kinase - phosphatidylinositol 3-kinase

PPP - Pentose Phosphate Pathway

PRPP - 5-Phosphoribosyl-1-Pyrophosphate

RDI - recommended daily intake

Retro - retroperitoneal

RIA - radioimmunoassay

ROS - reactive oxygen species

SAS - statistical analysis system

SEM - standard error of the mean

SPSS - statistical package for the social sciences

T2DM - Type 2 Diabetes Mellitus

TQEH - the Queen Elizabeth Hospital

UDP-N-GlcNAc - N-acetylglucosaminyl-1-phosphotransferase