

Developmental programming of allergic susceptibility

Amy Louise Wooldridge, BSc (Hons)

Early Origins of Health and Disease Research Group

Robinson Research Institute

Department of Obstetrics and Gynaecology

Adelaide School of Medicine

Faculty of Health Sciences

University of Adelaide

South Australia

Thesis submitted to the University of Adelaide for fulfilment of the requirements

for admission to the degree of Doctor of Philosophy (PhD)

December 2016



THE UNIVERSITY

of ADELAIDE

"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes."

-Marcel Proust

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ABSTRACT

Allergic susceptibility is associated with early life exposures, including intrauterine growth restriction and maternal allergy. Epidemiological and animal model studies suggest that restricted growth before birth is protective against later allergy development, whilst maternal allergy is generally associated with increased allergy risk in progeny. Causality and mechanisms mediating these associations are poorly understood, and I therefore investigated immune and allergic responses in ovine models following these prenatal exposures.

The first aim of study one (**chapter 2**) was to determine the effects of intrauterine growth restriction, due to placental restriction (PR), on allergic susceptibility. The second aim (**chapter 3**) was to determine the effects of maternal dietary methyl donor and cofactor supplementation during late pregnancy on allergic susceptibility of PR progeny, since methyl donors can regulate gene methylation via the one-carbon pathway. Placental restriction was induced by pre-pregnancy surgical reduction of placental attachment sites and its effects on progeny immune function and underlying mechanisms were investigated. Allergen-induced antibody and cutaneous hypersensitivity responses were measured in progeny from control and PR pregnancies following sensitisation to house dust mite and ovalbumin allergens. Effects of PR on cutaneous hypersensitivity responses did not correspond with effects on allergen-specific IgE responses. Delayed-phase cutaneous responses to ovalbumin were reduced in PR compared to control singletons, consistent with reports of epidemiological studies where low birth weight or poor fetal growth are generally protective against allergy, and despite no loss of IgE antibody response. Delayed-phase cutaneous responses to house dust mite were normal in PR singletons, despite enhanced IgE responses. Maternal dietary methyl donor and cofactor supplementation decreased antibody responses to allergens in some subgroups, but not those in which PR reduced cutaneous responses. This discord between antibody and cutaneous hypersensitivity responses suggests that mast cell function or other factors contribute to prenatally programmed regulation of allergy.

The aim of study two (**chapter 4**) was to investigate the effects of maternal allergic asthma on the fetal immune system in an ovine model. Maternal allergic asthma reduced relative fetal size and lung development in late gestation, but did not alter fetal immune tissue weights. In late gestation we detected an increase in thymocyte CD44 expression in fetuses from allergic compared to control ewes, suggestive of increased thymocyte activation.

In conclusion, maternal dietary supplementation with methyl donors and cofactors partially reversed the protective effects of restricted fetal growth against allergy, consistent with an epigenetic mechanism contributing to prenatal programming of allergic phenotype. Further research should include direct measures of one-carbon metabolism and methylation of immune-regulatory genes after PR and methyl donor supplementation, and of mast cell function as a potential mechanism for altered skin inflammatory responses to allergens. Results in the ovine model of maternal allergic asthma suggest that altered immune development may contribute to associations between maternal asthma and increased risk of allergy in progeny observed in human cohorts. The findings in this thesis provide direct evidence that allergic susceptibility can be programmed before birth.

STATEMENT OF ORIGINALITY AND AUTHENTICITY

I 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works. The following manuscripts have been accepted/published from this work:

- **Wooldridge AL**, Bischof RB, Meeusen EN, Liu H, Heinemann GK, Hunter DS, Kind KL, Owens JA, Clifton VL & Gatford KL. (2014) Placental restriction of fetal growth reduces cutaneous responses to antigen after sensitization in sheep, published by Am J Physiol Regul Integr Comp Physiol 306(7):R441-6. doi: 10.1152/ajpregu.00432.2013 (Appendix 1)

- Clifton VL, Moss TJ, **Wooldridge AL**, Gatford KL, Liravi B, Kim D, Mühlhäusler BS, Morrison JL, Davies A, De Matteo R, Wallace MJ, Bischof RJ. (2016) Development of an experimental model of maternal allergic asthma during pregnancy, published by J Physiol 594(5):1311-1325. doi: 10.1113/JP270752 (Appendix 2)
- **Wooldridge AL**, McMillan M, Marshall HS, Gatford KL. (2016) Systematic review protocol: relationship between fetal growth rate and postnatal allergy, published by JBI Database System Rev Implement Rep 14(11):11-20. doi: 10.11124/JBISRIR-2016-003177 (Appendix 3)
- Gatford KL, **Wooldridge AL**, Bischof RJ, Clifton VL, Kind KL. Pre-birth origins of allergy and asthma, accepted by J Reprod Immunol since PhD submission (Appendix 4)

Signed,

Amy Louise Wooldridge

ACKNOWLEDGEMENTS

Thank you to my four supervisors, Dr Kathryn Gatford, Dr Robert Bischof, A/Prof Vicki Clifton and Dr Karen Kind, for their continued support and advice throughout my candidature. Your extensive career advice and encouragement has been invaluable! My only regret from this PhD is that I was unable to submit my thesis within the time limit required for one of my supervisors to skydive with me.

Thanks also to the many members of my lab group and department, sharing their wisdom, jokes and own PhD journeys with me. Gary Heinemann, whose wisdom (including technical lab tips) I have quoted to many of my juniors already. Jessica Laurence, who fixed computer issues simply by looking at the computer whilst I tried to replicate them before her, who introduced me to a research leader at a conference when I was too afraid to approach them in person prior to my arranged visit to their laboratory and who had a calming presence even in the most trying of times. Rebecca Wilson and Benjamin Mayne for office antics (not limited to the “box person” decoy – passed as a real person on several occasions). Fellow sheep-wranglers – Hong Liu, Damien Hunter, Manpreet Kaur, Jen Rice, Anna Le Ber, Danila Marini, Helen Brodie. Those who gave advice on lab techniques – Hui Lu, Courtney McDonald and Dylan McCullough. To those who gave me career advice or have been my role models/mentors, knowingly or not – Tim Moss, Hannah Brown, Sarah Robertson, Claire Roberts, and the graduate research coordinator from another university, who enthusiastically emphasised the importance of completing the literature review with respect to candidature completions. This advice was given at a conference dinner, upon finding out that my literature review was not yet completed. Thanks also to my friends from my undergraduate degree for their advice and support as they progress in their own research careers in different locations across Australia.

I am forever thankful to my parents for housing and feeding me throughout much of my PhD, in addition to my whole family's helpful sending of memes related to finishing a PhD at the age of 90 years. I hereby proclaim that it is now safe to ask me whether I've finished writing my thesis.

I am very grateful to have received an Australian Postgraduate Award and a Healthy Development Adelaide and Channel 7 Children's Research Foundation supplementary scholarship to support me during my PhD candidacy. I am also very grateful to have been awarded the Robert Seamark Scholarship in Obstetrics and Gynaecology from the University of Adelaide, in addition to a travel grants from Healthy Development Adelaide, the School of Paediatrics and Reproductive Health/Robinson Research Institute, the Perinatal Society of Australia and New Zealand and the Endocrine Society of Australia. Without these travel awards, presenting the research in this thesis at conferences would not have been possible, my research CV would have been poor, and my confidence at networking would still be sorely lacking. My involvement in the publications listed in the following pages that do not form a part of this thesis has given me considerable confidence and skills in collaborative work, more experience in the peer-review and publication processes, and has helped me greatly to expand my professional network and reading outside of my primary field. The skills that I have gained through these other studies have already proven to be useful.

The work presented in Chapters 2 and 3 was supported by project grants from the National Health and Medical Research Council of Australia (ID nos. 627123 and 1011767). Thank you to the staff of Laboratory Animal Services, University of Adelaide, for their excellence in animal care, to Dr Martin Elhay, Pfizer Animal Health, Parkville, Australia for provision of Clostridial Ig ELISA reagents and Evonik Degussa GmbH, Hanau, Germany, for donating the rumen-protected methionine (Mepron) used in this study. The work presented in Chapter 4 was supported by the Jack Brockhoff Foundation (Grant no. 3699) and the Victorian Government Operational Infrastructure Support Program. Thank you to the staff of Monash Animal Services for care of the animals. Thank you to the administration staff for care of Higher Degree by Research students like me!

TABLE OF ABBREVIATIONS

ABC Study	Auckland Birthweight Collaborative Study
AF647	Alexa Fluor® 647
AGA	Adequate size for gestational age
ANOVA	Analysis of variance
BAL	Bronchoalveolar lavage
CD	Cluster of differentiation
cf.	<i>Confer</i>
CON	Control
dGA	Days gestational age
DNA	Deoxyribonucleic acid
DOHaD	Developmental origins of health and disease
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
Et1	Endothelin-1
FACS	Fluorescence-activated cell sorting
FITC	Fluorescein isothiocyanate
FOXP3	Forkhead box P3
FSC	Forward-scatter
HBSS	Hank's buffered saline solution
HBW	High birth weight
HDM	House dust mite
HMD	High methyl donor and cofactors diet
HRP	Horseradish peroxidase
IgA	Immunoglobulin type A

IgE	Immunoglobulin type E
IgG	Immunoglobulin type G
IgG₁	Immunoglobulin type G ₁
IgM	Immunoglobulin type M
ISAAC	International Study of Asthma and Allergies in Childhood
IU	International units
IUGR	Intrauterine growth restriction
LBW	Low birth weight
LIFT Study	Loire Infant Follow-Up Study
LMD	Low methyl donor and cofactors diet
LPS	Lipopolysaccharide
M:F	Male:female
mAbs	Monoclonal antibodies
MB	Multiple birth (twin or triplet)
MHC I	Major histocompatibility complex class I
MHC II	Major histocompatibility complex class I
mo	Months old
NBW	Normal birth weight
NTD	Neural tube defect
OR	Odds ratio
OVA	Ovalbumin
PAULA Study	Perinatal Asthma and Environment Long-term Allergy Study
PE	Phycoerythrin
PR	Placental restriction or placentally-restricted

PR+METHYL, PR+M	Placentally-restricted, maternal dietary methyl donor and cofactor-supplemented
RBC	Red blood cell
RR	Risk ratio
RUNX3	Runt-related transcription factor 3
SAGE	Study of Asthma Genes and the Environment
SB	Singleton birth
SE	Standard error of the mean
SGA	Small for gestational age
SPT	Skin prick test
SSC	Side-scatter
Th1	Type 1 T helper
Th2	Type 2 T helper
TMB	3',3',5',5'-tetramethyl-benzidine dihydrochloride hydrate
Total Ig	Total immunoglobulin
Treg	Regulatory T cell
WBC	White blood cell
yo	Years old

MANUSCRIPTS ARISING FROM PhD

Work directly related to this thesis:

Published manuscripts:

Wooldridge AL, Bischof RB, Meeusen EN, Liu H, Heinemann GK, Hunter DS, Kind KL, Owens JA, Clifton VL & Gatford KL. (2014) Placental restriction of fetal growth reduces cutaneous responses to antigen after sensitization in sheep, *Am J Physiol Regul Integr Comp Physiol* 306(7):R441-6. doi: 10.1152/ajpregu.00432.2013 (Appendix 1)

Clifton VL, Moss TJ, **Wooldridge AL**, Gatford KL, Liravi B, Kim D, Mühlhäusler BS, Morrison JL, Davies A, De Matteo R, Wallace MJ, Bischof RJ. (2016) Development of an experimental model of maternal allergic asthma during pregnancy, *J Physiol* 594(5):1311-1325. doi: 10.1113/JP270752 (Appendix 2)

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Accepted manuscripts:

Gatford KL, **Wooldridge AL**, Bischof RJ, Clifton VL, Kind KL. Pre-birth origins of allergy and asthma, accepted by *J Reprod Immunol* since PhD submission (Appendix 4)

Other manuscripts published during PhD:

Liu H, Schultz CG, De Blasio MJ, Peura AM, Heinemann GK, Harryanto H, Hunter DS, **Wooldridge AL**, Kind KL, Giles LC, Simmons RA, Owens JA, Gatford KL. (2015) Effect of placental restriction and neonatal exendin-4 treatment on postnatal growth, adult body composition and in vivo glucose metabolism in the sheep, *Am J Physiol Endocrinol Metab* 309(6):E589-600. doi:

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(invited symposium presentation, KL Gatford)

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(oral-poster presentation, AL Wooldridge)

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(poster presentation, AL Wooldridge)

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(oral presentation, AL Wooldridge)

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(oral presentation, AL Wooldridge)

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(oral presentation, AL Wooldridge)

Wooldridge AL, Bischof RJ, Meeusen EN, Liu H, Heinemann GK, Hunter DS, Kind KL, Owens JA, Clifton VL, Gatford KL. (2013). Does late pregnancy methyl donor supplementation reverse effects of placental restriction on immune function in sheep? *Robinson Institute Research Symposium, Adelaide, Australia*
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(poster presentation, AL Wooldridge)

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(oral presentation, AL Wooldridge)