Developmental programming of allergic susceptibility

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

-Marcel Proust
# TABLE OF CONTENTS

TABLE OF CONTENTS .................................................................................................................. 2
LIST OF TABLES AND FIGURES .................................................................................................. 7
ABSTRACT ........................................................................................................................................ 8
STATEMENT OF ORIGINALITY AND AUTHENTICITY .................................................................. 10
ACKNOWLEDGEMENTS ............................................................................................................... 12
TABLE OF ABBREVIATIONS ....................................................................................................... 14
MANUSCRIPTS ARISING FROM PhD ......................................................................................... 17
  Work directly related to this thesis: ............................................................................................ 17
  Other manuscripts published during PhD: .................................................................................. 18
CONFERENCE ABSTRACTS ARISING FROM PhD ................................................................. 20
Chapter 1: INTRODUCTION/LITERATURE REVIEW ................................................................. 24
  1.1 Overview ............................................................................................................................... 24
  1.2 Statements of authorship for Chapter 1 ................................................................................ 25
    1.2.1 Systematic review protocol: relationship between fetal growth rate and postnatal allergy ................................................................................................................................. 25
    1.2.2 Pre-birth origins of allergy and asthma ......................................................................... 27
  1.3 Introduction ............................................................................................................................ 29
    1.3.1 General introduction and scope of literature review ....................................................... 29
    1.3.2 Developmental origins of health and disease (DOHaD) concepts ............................ 30
      1.3.2.1 Introduction to DOHaD concepts ........................................................................... 30
      1.3.2.2 Application of DOHaD concepts to improve human health ............................... 31
    1.3.3 Intrauterine growth restriction (IUGR) definitions and incidence ............................ 33
    1.3.4 Allergy definitions and incidence .................................................................................. 34
    1.3.5 Incidence of maternal allergy and asthma ................................................................. 36
1.4 Developmental programming of allergic disease in humans.................................................. 36

1.4.1 Associations between low birth weight or poor fetal growth and offspring allergy .......... 36
1.4.2 Evidence from human cohorts for maternal asthma and allergy during pregnancy as allergy risk factors .......................................................................................................................... 52
1.4.3 Evidence from human cohorts for methyl donor abundance as an asthma and allergy risk factor .................................................................................................................................. 54
1.4.4 Strengths and limitations of human studies ..................................................................... 56
   1.4.4.1 Strengths ................................................................................................................................. 56
   1.4.4.2 Limitations ................................................................................................................................. 57

1.5 Developmental programming of allergic disease in animal models .................................. 60

1.5.1 IUGR ........................................................................................................................................ 60
   1.5.1.1 Chronic experimental IUGR reduces allergic sensitisation .............................................. 60
   1.5.1.2 Limitations in existing studies of experimental IUGR and allergic outcomes .............. 62
1.5.2 Maternal allergy and asthma ................................................................................................. 62
   1.5.2.1 Experimental allergy and asthma in the mother pre-dispose progeny to allergy .......... 63
   1.5.2.2 Limitations in existing studies of experimental maternal allergy and asthma .......... 65
1.5.3 One-carbon pathways ........................................................................................................... 66
   1.5.3.1 Experimental manipulation of one-carbon pathways and progeny allergy .............. 66
   1.5.3.2 Limitations in existing studies of one-carbon pathways and progeny allergy .......... 67

1.6 Thesis hypotheses and aims .................................................................................................. 68

Chapter 2: Effect of placental restriction on susceptibility to allergy ........................................... 69

2.1 Overview ................................................................................................................................... 69
2.2 Statement of authorship – Placental restriction of fetal growth reduces cutaneous responses to antigen after sensitization in sheep ........................................................................... 70
2.4 Introduction .................................................................................................................. 74
2.5 Methods ....................................................................................................................... 75
  2.5.1 Animal model .......................................................................................................... 75
  2.5.2 Immunisation, sensitisation, and cutaneous hypersensitivity testing ...................... 76
  2.5.3 Serum antibody concentrations ............................................................................. 76
  2.5.4 Cell counts .............................................................................................................. 77
  2.5.5 Statistical analysis ................................................................................................. 78
2.6 Results .......................................................................................................................... 78
  2.6.1 Birth weight and gestational age ........................................................................... 78
  2.6.2 Circulating immune cells ....................................................................................... 78
  2.6.3 Antibody responses to house dust mite (HDM) allergen and ovalbumin (OVA) sensitisation ................................................................................................................ 79
  2.6.4 Antibody responses to Clostridial vaccination ......................................................... 81
  2.6.5 Correlation between birth weight and cutaneous histamine responses ............... 82
2.7 Discussion ..................................................................................................................... 82
2.8 Perspectives and Significance ...................................................................................... 86

Chapter 3: Evidence for an epigenetic process for perinatal programming of allergy: maternal dietary methyl donor and cofactor supplementation through late gestation partially reverses protection against allergic sensitisation in an ovine model of IUGR ........................................... 88
  3.1 Overview .................................................................................................................... 88
  3.2 Introduction ................................................................................................................ 89
  3.3 Materials and Methods ............................................................................................. 91
    3.3.1 Animal model ....................................................................................................... 91
    3.3.2 Immunisation, sensitisation and cutaneous hypersensitivity testing .................... 92
    3.3.3 Circulating blood cell counts and serum antibody concentrations ..................... 93
3.3.4 Quantitation of intradermal mast cells .......................................................... 93
3.3.5 Statistical analysis ......................................................................................... 94
3.4 Results ............................................................................................................. 95
3.4.1 Birth phenotype and gestation length ......................................................... 95
3.4.2 Circulating red and white blood cell counts ............................................... 99
3.4.3 Antibody responses .................................................................................... 101
3.4.4 Cutaneous hypersensitivity responses ....................................................... 104
3.4.5 Mast cell density ........................................................................................ 106
3.5 Discussion ....................................................................................................... 107

Chapter 4: Effects of maternal asthma on the fetal immune system .................. 114
4.1 Overview ......................................................................................................... 114
4.2 Statement of authorship form – Development of an experimental model of maternal allergic asthma during pregnancy .......................................................... 115
4.3 Introduction and experimental paradigm ....................................................... 118
4.4 Methods .......................................................................................................... 121
4.4.1 Animals and experimental design ............................................................... 121
4.4.2 Endoscopic airway challenges .................................................................. 124
4.4.3 Post-mortem and tissue collection ............................................................. 124
4.4.4 Flow cytometry .......................................................................................... 124
4.4.5 Statistical analyses ..................................................................................... 126
4.5 Results ............................................................................................................. 128
4.5.1 Post-mortem organ weights ....................................................................... 128
4.5.2 Fetal antibodies to HDM allergen ............................................................... 130
4.5.3 Fetal immune cell phenotype ..................................................................... 130
4.6 Discussion ....................................................................................................... 131

Chapter 5: General discussion ........................................................................... 136
5.1 Introduction ................................................................................................................................. 136
5.2 Developmental programming of allergic susceptibility ............................................................. 137
5.3 Strengths and limitations of the studies in this thesis ............................................................... 140
5.5 Conclusion .................................................................................................................................. 142
REFERENCES .................................................................................................................................... 143
APPENDICES ....................................................................................................................................... 160
Appendix 1 Placental restriction of fetal growth reduces cutaneous responses to antigen after
sensitization in sheep ...................................................................................................................... 160
Appendix 2 Development of an experimental model of maternal allergic asthma during pregnancy
........................................................................................................................................................................... 161
Appendix 3 Systematic review protocol: relationship between fetal growth rate and postnatal
allergy .......................................................................................................................................................... 162
Appendix 4 Pre-birth origins of allergy and asthma ........................................................................ 163
Appendix 5 Effect of placental restriction and neonatal exendin-4 treatment on postnatal growth,
adult body composition and in vivo glucose metabolism in the sheep ............................................ 164
Appendix 6 In utero programming of allergic susceptibility ............................................................. 165
Appendix 7 Placental restriction in multi-fetal pregnancies increases spontaneous ambulatory
activity during daylight hours in young adult female sheep ........................................................... 166
Appendix 8 A review of fundamental principles for animal models of DOHaD research: an
Australian perspective .......................................................................................................................... 167
Appendix 9 Placental restriction in multi-fetal pregnancies and between-twin differences in size at
birth alter neonatal feeding behaviour in the sheep ............................................................................. 168
LIST OF TABLES AND FIGURES

Table 1.1 Summary of key studies for effects of weight/size at birth on allergic diseases

Figure 2.1 In vivo study timeline

Figure 2.2 Serum antibody responses to sensitisation

Figure 2.3 Relationship between birth weight and skin wheal response to histamine

Table 3.1 Effect of placental restriction (PR) and late pregnancy maternal dietary methyl donor and cofactor supplementation on body size at birth

Figure 3.1 Proportion of positive and negative responders at 24 h after intradermal challenge with ovalbumin

Table 3.2 Effect of sex on circulating white blood cell subsets at 33 weeks of age

Table 3.3 Effect of PR on the proportion of antibody responses to house dust mite

Figure 3.2 A. Upper dermis of skin sections from adult sheep stained with toluidine blue for mast cells. B. Upper dermis mast cell density in singleton birth and multiple birth male and female sheep

Figure 4.1 Study design

Figure 4.2 Gating strategies - representative fluorescence-activated cell sorting (FACS) profiles from the spleen of an individual sheep

Table 4.1 Antibodies used to stain cell receptors for flow cytometry

Table 4.2 Placental phenotype and maternal and fetal weights at post-mortem

Figure 4.3 Percentage of lymphocytes positive for cluster of differentiation (CD)44 expression from fetal thymus and spleen
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:


• 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
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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.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>ABC Study</td>
<td>Auckland Birthweight Collaborative Study</td>
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<tr>
<td>AF647</td>
<td>Alexa Fluor® 647</td>
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<tr>
<td>AGA</td>
<td>Adequate size for gestational age</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
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<td>CD</td>
<td>Cluster of differentiation</td>
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<td>cf.</td>
<td>Confer</td>
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<tr>
<td>CON</td>
<td>Control</td>
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<td>dGA</td>
<td>Days gestational age</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOHaD</td>
<td>Developmental origins of health and disease</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>Et1</td>
<td>Endothelin-1</td>
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<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorting</td>
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<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
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<td>FOXP3</td>
<td>Forkhead box P3</td>
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<tr>
<td>FSC</td>
<td>Forward-scatter</td>
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<tr>
<td>HBSS</td>
<td>Hank's buffered saline solution</td>
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<td>HBW</td>
<td>High birth weight</td>
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<td>HDM</td>
<td>House dust mite</td>
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<td>HMD</td>
<td>High methyl donor and cofactors diet</td>
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<td>HRP</td>
<td>Horseradish peroxidase</td>
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<td>IgA</td>
<td>Immunoglobulin type A</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>IgE</td>
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<tr>
<td>ISAAC</td>
<td>International Study of Asthma and Allergies in Childhood</td>
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<tr>
<td>IU</td>
<td>International units</td>
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<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
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<td>LBW</td>
<td>Low birth weight</td>
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<tr>
<td>LIFT Study</td>
<td>Loire Infant Follow-Up Study</td>
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<tr>
<td>LMD</td>
<td>Low methyl donor and cofactors diet</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>M:F</td>
<td>Male:female</td>
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<tr>
<td>mAbs</td>
<td>Monoclonal antibodies</td>
</tr>
<tr>
<td>MB</td>
<td>Multiple birth (twin or triplet)</td>
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<tr>
<td>MHC I</td>
<td>Major histocompatibility complex class I</td>
</tr>
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<td>Major histocompatibility complex class I</td>
</tr>
<tr>
<td>mo</td>
<td>Months old</td>
</tr>
<tr>
<td>NBW</td>
<td>Normal birth weight</td>
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<tr>
<td>NTD</td>
<td>Neural tube defect</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<td>Ovalbumin</td>
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<td>PAULA Study</td>
<td>Perinatal Asthma and Environment Long-term Allergy Study</td>
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<tr>
<td>PE</td>
<td>Phycoerythrin</td>
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<tr>
<td>PR</td>
<td>Placental restriction or placentally-restricted</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-------------</td>
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<tr>
<td>PR+METHYL, PR+M</td>
<td>Placentally-restricted, maternal dietary methyl donor and cofactor-supplemented</td>
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<td>RBC</td>
<td>Red blood cell</td>
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<td>RR</td>
<td>Risk ratio</td>
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<td>RUNX3</td>
<td>Runt-related transcription factor 3</td>
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<td>SAGE</td>
<td>Study of Asthma Genes and the Environment</td>
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<tr>
<td>SB</td>
<td>Singleton birth</td>
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<tr>
<td>SE</td>
<td>Standard error of the mean</td>
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<td>White blood cell</td>
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MANUSCRIPTS ARISING FROM PhD

Work directly related to this thesis:

Published manuscripts:


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:**


CONFERENCE ABSTRACTS ARISING FROM PhD


**Wooldridge AL, Gatford KL, Moss TJ, McDonald C, Clifton VL, Bischof RJ.** (2016). Effects of maternal asthma on the fetal immune system, *Fetal and Neonatal Workshop, Magnetic Island, Australia* (oral presentation, AL Wooldridge)


(oral presentation, M Kaur)


(post poster presentation, AL Wooldridge)


(post poster presentation, M Kaur)


(post poster presentation, AL Wooldridge)


(oral presentation, AL Wooldridge)

(poster 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? *Faculty of Health Sciences Postgraduate Conference, Adelaide, Australia*
(poster presentation, AL Wooldridge)

(oral presentation, AL Wooldridge)