The Effect of Maternal Asthma During Pregnancy on Placental Function and Fetal Development

Vanessa Evonne Murphy
B Med Chem (Hons)

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The University of Newcastle, Australia
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Declaration

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution

Signed ......................................

Vanessa E Murphy
January 2004
Acknowledgements

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Philippians 4:13

I can do all things through Christ who strengthens me
Publication List

The work presented in this thesis has directly resulted in the following publications:


2. **Murphy VE** and Clifton VL. Alterations in human placental 11β-hydroxysteroid dehydrogenase type 1 and 2 with gestational age and labour. Placenta 2003; 24(7), 739-744.


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3. **Murphy VE**, Zakar T, Smith R, Giles WB, Gibson PG and Clifton VL. The effects of asthma during pregnancy on placental 11β-hydroxysteroid dehydrogenase type 2 and neonatal birth weight centile. 10th NSW Scientific
Meeting of the Australian Society for Medical Research, Sydney, Australia, 4 June 2001 (Oral).

4. **Murphy VE**, Zakar T, Smith R, Giles WB, Gibson PG and Clifton VL. A potential mechanism for growth retardation in pregnancies complicated by asthma. 44th Annual Scientific Meeting of the Endocrine Society of Australia, Gold Coast, Australia, 9-12 September 2001 (Oral - Finalist for the Novartis Junior Scientist Award).

5. Clifton VL, **Murphy VE**, Giles WB, Zakar T, Gibson PG and Smith R. 11β-hydroxysteroid dehydrogenase type 2 activity in pregnancies complicated by asthma. 8th Meeting of the International Federation of Placenta Associations, Sorrento, Italy, 19-23 September 2001 (Presented by Dr Vicki Clifton).

6. **Murphy VE**, Zakar T, Smith R, Giles WB, Gibson PG and Clifton VL. Female fetal growth is adversely affected by maternal asthma in the absence of glucocorticoid therapy. 40th National Scientific Conference of the Australian Society for Medical Research, Gold Coast, Australia, 24-27 November 2001 (Poster - won the Medibank Private Student Researcher Award).


8. Clifton VL, **Murphy VE**, Zakar T, Smith R, Giles WB and Gibson PG. Female fetal growth is adversely affected by maternal asthma in the absence of inhaled glucocorticoid (ICS) therapy. 98th Annual Scientific Meeting of the American Thoracic Society, Atlanta, GA, USA, 17-22 May 2002 (Presented by Prof Peter Gibson).

9. **Murphy VE**, Gibson PG, Giles WB, Zakar T, Smith R, Kessell CG and Clifton VL. Maternal asthma affects female fetal growth and is associated with reduced placental 11β-HSD2 activity and altered sensitivity to cortisol. 11th NSW Scientific Meeting of the Australian Society for Medical Research, Sydney, Australia, 3 June 2002 (won Best Poster Presentation by a Student).


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### Abbreviations

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<th>Description</th>
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<tr>
<td>11β-HSD</td>
<td>11 beta-hydroxysteroid dehydrogenase</td>
</tr>
<tr>
<td>2D-PAGE</td>
<td>Two dimensional-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>³H</td>
<td>Tritium (Tritiated)</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AME</td>
<td>Apparent mineralocorticoid excess</td>
</tr>
<tr>
<td>AMS</td>
<td>Asthma Management Service</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>β₂-agonist</td>
<td>Beta two adrenergic receptor agonist</td>
</tr>
<tr>
<td>BDP</td>
<td>Beclomethasone dipropionate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BPD</td>
<td>Biparietal diameter</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>BWC</td>
<td>Birth weight centile</td>
</tr>
<tr>
<td>C section</td>
<td>Caesarean section</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic 3',5'-adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic 3',5'-guanosine monophosphate</td>
</tr>
<tr>
<td>CHAPS</td>
<td>3-[(3-chloramidopropyl)-dimethylammonio]-1-propanesulfonate</td>
</tr>
<tr>
<td>CHCA</td>
<td>α-cyano-4-hydroxycinnamic acid</td>
</tr>
<tr>
<td>cpm</td>
<td>Counts per minute</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
</tr>
<tr>
<td>Cₜ</td>
<td>Threshold cycle</td>
</tr>
<tr>
<td>Da</td>
<td>Daltons</td>
</tr>
<tr>
<td>dATP</td>
<td>2'-deoxyadenosine 5'-triphosphate</td>
</tr>
<tr>
<td>dCTP</td>
<td>2'-deoxycytidine 5'-triphosphate</td>
</tr>
<tr>
<td>dGTP</td>
<td>2'-deoxyguanosine 5'-triphosphate</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>Dehydroepiandosterone sulfate</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Deoxynucleotide triphosphates</td>
</tr>
<tr>
<td>dpm</td>
<td>Disintegrations per minute</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>dTTP</td>
<td>2'-deoxythymidine 5'-triphosphate</td>
</tr>
<tr>
<td>dUTP</td>
<td>2'-deoxyuridine 5'-triphosphate</td>
</tr>
<tr>
<td>EAM</td>
<td>Energy absorbing molecule</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene-diamine-tetra-acetic acid</td>
</tr>
<tr>
<td>EGTA</td>
<td>Ethylene-glycol-bis(β-aminoethyl ether)-tetra-acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume at one second</td>
</tr>
<tr>
<td>FRI</td>
<td>Ferring Research Institute</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>fwd</td>
<td>Forward (primer)</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte macrophage-colony stimulating factor</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>HC:AC</td>
<td>Head circumference to abdominal circumference ratio</td>
</tr>
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</table>
hCG  Human chorionic gonadotropin
HPA  Hypothalamic-pituitary-adrenal
HPLC  High performance liquid chromatography
IFN-γ  Interferon gamma
IgE  Immunoglobulin E
IGF  Insulin-like growth factor
IGFBP  Insulin-like growth factor binding protein
IL  Interleukin
IMAC  Immobilised metal affinity capture
IQ  Intelligence quotient
IU  International units
IUGR  Intrauterine growth restriction
KCl  Potassium chloride
Km  Michaelis constant
LPS  Lipopolysaccharide
LTB₄  Leukotriene B₄
MALDI  Matrix assisted laser desorption/ionisation
MR  Mineralocorticoid receptor
mRNA  Messenger RNA
MS  Mass spectrometry
MW  Molecular weight
m/z  Mass to charge ratio
n  Number of subjects
NaCl  Sodium chloride
NAD  Nicotinamide adenine dinucleotide
NADH  Nicotinamide adenine dinucleotide reduced form
NADP  Nicotinamide adenine dinucleotide phosphate
NADPH  Nicotinamide adenine dinucleotide phosphate reduced form
NBT/BCIP  Nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate
Ni-NTA  Nickel nitrilotriacetic acid
No.  Number
NO  Nitric oxide
NTC  No template control
PBS  Phosphate buffered saline
PCA  Principal component analysis
PCR  Polymerase chain reaction
PCO₂  Partial pressure of carbon dioxide
PEF  Peak expiratory flow
PEFR  Peak expiratory flow rate
PGE₂  Prostaglandin E₂
PGF₂α  Prostaglandin F₂α
pI  Isoelectric point
PIH  Pregnancy induced hypertension
PO₂  Partial pressure of oxygen
PPROM  Preterm premature rupture of membranes
PVDF  Polyvinylidene fluoride
RDS  Respiratory distress syndrome
rev  Reverse (primer)
RIA  Radioimmunoassay
rRNA  Ribosomal RNA
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase-polymerase chain reaction</td>
</tr>
<tr>
<td>SAX</td>
<td>Strong anion exchange</td>
</tr>
<tr>
<td>SD</td>
<td>Systolic/diastolic ratio</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SELDI-TOF</td>
<td>Surface enhanced laser desorption/ionisation-time of flight</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SPA</td>
<td>Sinapinic acid</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris borate EDTA</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris buffered saline</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor-beta</td>
</tr>
<tr>
<td>Th1/2</td>
<td>T helper type 1/2</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>TOF</td>
<td>Time of flight</td>
</tr>
<tr>
<td>TTTS</td>
<td>Twin to twin transfusion syndrome</td>
</tr>
<tr>
<td>TX-100</td>
<td>Triton X-100</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
</tr>
<tr>
<td>WCX</td>
<td>Weak cation exchange</td>
</tr>
</tbody>
</table>
Abstract

Maternal asthma is associated with low birth weight, a risk factor for disease in adult life. To determine the mechanisms involved, the relationships between mother, placenta and fetus were examined in asthmatic and non-asthmatic pregnancies.

Maternal asthma and its treatment (no glucocorticoid or glucocorticoid) was monitored throughout pregnancy. Fetal growth was examined during gestation, and at birth, neonatal size and sex were determined. Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme activity and umbilical vein plasma cortisol and estriol concentrations were measured. Placental cytokine, growth factor and glucocorticoid receptor (GR) mRNA were determined using quantitative RT-PCR.

Birth weight of female neonates in the no glucocorticoid asthmatic group only, was significantly reduced compared to females of the non-asthmatic group. Male neonates were unaffected by asthma or its treatment. Asthmatic women pregnant with a female fetus showed a significant increase in circulating monocytes and glucocorticoid treatment as pregnancy progressed, while those pregnant with a male fetus did not, suggesting that maternal asthma worsens in the presence of a female fetus. 11β-HSD2 activity was significantly reduced in placentae from female neonates of the no glucocorticoid group compared to other female neonates and was associated with a trend towards higher plasma cortisol, reduced fetal adrenal activity demonstrated by lower cord blood estriol, reduced placental GR expression, no alteration in placental or fetal insulin-like growth factors or their binding proteins and a significantly increased Th2:Th1 cytokine mRNA ratio, which was inversely correlated with 11β-HSD2 activity in all females. Reduced placental 11β-HSD2 activity may be an important component leading to decreased female fetal growth in pregnancies complicated by asthma.

This study provides strong evidence for a fetal sex-specific effect on the maternal immune system which can have adverse effects on the female fetus. The female fetus alters maternal inflammatory pathways, which when not controlled by the use of inhaled glucocorticoids results in reduced placental 11β-HSD2 activity, contributing to suppressed fetal adrenal function and a late gestation decrease in female fetal growth.