# Maternal Probiotic Intervention as a Prophylaxis against the Impact of Neonatal Stress:

# Implications for Irritable Bowel Syndrome

By

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MSc (Food Science and Technology)

A Thesis Submitted in Total Fulfilment of the Requirements for the Degree of

**Doctor of Philosophy**

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February 2013

# STATEMENT OF ORIGINALITY

*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 to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.*

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# ACKNOWLEDGMENTS

This thesis was made possible due to the many people who have provided unending support throughout my PhD candidature. Firstly, I would like to acknowledge my supervisor Associate Professor Deborah Hodgson for her tireless support and guidance. What I have learned and the person I have become because of her encouragement, enthusiasm and leadership cannot be expressed in words. I am most grateful to have not only acquired an amazing supervisor but a dear friend as well. Thank you Deb.

I would like also to thank my former co-supervisor Dr Michelle Adams for her support in the early stage of my PhD journey.

I would like to thank Mrs Elly Huber and Miss Donna Catford for their dedicated animal care, patient animal husbandry, and unswerving dedication to the research process.

Special thanks to Professor Mick Hunter, Dr Miles Bore and all the people in the School of Psychology for adopting me and for their ongoing support. I would like to specially thank Mrs Lynne Brunt, Mrs Sandra Dimmock and Mr Jeff Drummond.

Special thanks to Professor Hugh Dunstan, Professor Scott Holmes and Mrs Karen Kincaid for their ongoing support.

I would also like to express my gratitude to Dr Trevor Moffiet and Mr Kim Colyvas for their assistance with statistical analyses and to Jane Todd for copy-editing my thesis.

I acknowledge the assistance provided to me by the Australian Federal Government and the University of Newcastle via the provision of my Endeavour International Postgraduate Research Scholarship and University of Newcastle Postgraduate Research Scholarship.

A huge thank you to Dr Tamo Nakamura and past and present PhD students specially Adam and Luba for their advice and insightful guidance but mostly for being such good friends.

I would like to thank my parents and family and my wife's family for their continued love, support and encouragement over the years.

More than anybody I would like to thank Mahta for all her patience, support and love.

## Publications arising from this thesis

### A. Peer reviewed journal paper

1. **Barouei, J.**, Adams, M. C. & Hodgson, D. M. (2009). Prophylactic Role of Maternal Administration of Probiotics in Intestinal and Immune Dysfunctions Induced by Neonatal stress. *Medical Hypotheses*, 73: 764-767.

2. **Barouei, J.**, Moussavi, M. & Hodgson, D. M. (2012). Effect of Maternal Probiotic Intake on HPA axis, Immunity and Gut Microbiota in a Rat Model of Irritable Bowel Syndrome. *PLoS ONE*, doi: 10.1371/journal.pone.0046051

3. **Barouei, J.**, Moussavi, M. & Hodgson, D. M. Maternal Probiotic Intervention Moderates Stress-induced Intestinal Associated Neuroendocrine Gene Expression in Adult Rat offspring (Submitted).

4. **Barouei, J.**, Moussavi, M. & Hodgson, D. M. Perinatal Maternal Probiotic Intervention Impacts Immune Responses and Ileal Mucin mRNA Expression in a Rat Model of Irritable Bowel Syndrome (Submitted).

### B. Peer reviewed conference abstracts

1- **Barouei, J.,** Moussavi, M. & Hodgson, D. M. (2012) Maternal Probiotic Intervention Protects Against Immune Alterations and Gut Dysfunctions in the Maternally Separated Rat Model of Irritable Bowel Syndrome. 4th American Society for Microbiology (ASM) Conference on Beneficial Microbes, October 22-26, 2012, San Antonio, Texas, USA. **(Oral Presentation)**

2- **Barouei, J.** & Hodgson, D. M. (2012). Maternal Probiotic Intervention Protects against Neuroendocrine and Immune Dysfunctions and Disruption of Gut Microflora Balance Provoked by Neonatal and Subsequent Adult Stress in Wistar Rats. Annual Meeting of the International Behavioral Neuroscience Society (IBNS), June 5-10, 2012, Kailua-Kona, Hawaii, USA, P142

3- **Barouei, J.** & Hodgson, D. M. (2011). Prophylactic potential of maternal probiotic supplementation against intestinal dysfunctions induced by early life stress. 7th World Congress on Developmental Origins of Health and Disease (DOHaD), September 18-21, 2011, Portland, Oregon, USA, *Journal of Developmental Origins of Health and Disease* **2** (Supp 1): S77

4- **Barouei, J.,** Adams, M. C. & Hodgson, D. M. (2011). Prophylactic effect of perinatal maternal probiotic intervention in brain-gut axis alterations provoked by early life stress. 18th Annual Meeting of The PsychoNeuroImmunology Research Society (PNIRS), June 8-11, 2011, Chicago, IL, USA, *Brain, Behavior, and Immunity* **25** (Supp 2): S189-S190

5- **Barouei, J.,** Adams, M. C. & Hodgson, D. M. (2010). Maternal probiotic intervention ameliorates endocrine and immune dysfunctions and restores unbalanced colonic flora induced by early life stress. 34th Annual Scientific Meeting of the Nutrition Society of Australia, November 30-Dec 4, 2010, Perth, Australia, *Australasian Medical Journal* **3** (12): 935 **(Oral Presentation)**

6- **Barouei, J.,** Adams, M. C. & Hodgson, D. M. (2010). Gender differentially influences the impact of neonatal and subsequent adult stress on gut integrity. 17th Annual Meeting of The PsychoNeuroImmunology Research Society (PNIRS), June 2-5, 2010, Dublin, Ireland, *Brain, Behavior, and Immunity* **24** (Supp 1): S61

7- **Barouei, J.,** Adams, M. C. & Hodgson, D. M. (2009). Maternal probiotic supplementation prevents stress induced unfavourable alterations in the balance of the enteric microflora. 33rd Annual Scientific Meeting of the Nutrition Society of Australia, p. 102, Newcastle, Australia, December 8-11, 2009. **(Oral Presentation)**

8- **Barouei J.,** Hodgson, D. M. & Adams M. C. (2009). Maternal supplementary probiotics protects against unfavourable neuroendocrine alterations provoked by neonatal maternal deprivation. 5$^{th}$ Probiotics, Prebiotics and New Foods Conference, 13-15 September 2009, Rome, Italy.

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## Thesis Summary

Neonatal stress is a common early life event, reported in some instances to be associated with adverse physiological alterations that persist into adulthood. This concept has been applied to the ontogeny of functional gastrointestinal disorders such as irritable bowel syndrome (IBS). The use of probiotics in IBS patients has emerged as a treatment approach to improve some IBS symptoms. In addition, new research in rodent models indicates that ***neonatal probiotic intervention*** may assist in the prevention of brain-gut axis dysfunctions believed to be associated with IBS. The aim of this study was to determine whether perinatal (both pre and post natal) maternal probiotic supplementation could act prophylactically to block endocrine, immune and gut dysfunctions in rats exposed to neonatal stress (maternal separation) either alone or in combination with adult exposure to stress. This model has been proposed to mimic most of the cardinal features of IBS.

The first series of studies (Chapter 3) examined the effect of maternal probiotic intervention on HPA-axis responses and gut-associated neuroendocrine function including analysis of mRNA expression of corticotropin releasing hormone receptors 1 and 2 (CRH-R1 and CRH-R2), and nerve growth factor (NGF). The results of the study revealed that maternal probiotic intervention induced activation of neonatal stress pathways as indicated by greatly enhanced corticosterone levels, which persisted into adulthood, and exacerbated ACTH responses to stress in adulthood. Maternal probiotic intervention affected gut-associated neuroendocrine gene expression profiles depending on age, gender and stress protocol. These effects include synergism, antagonism and normalisation.

The second series of studies (Chapter 4) examined the effect of maternal probiotic intervention on systemic and gut-associated immune functions. In this chapter plasma levels of cytokines IFN-γ, TNF-α and IL-6, plasma Haptoglobin and IgA, and luminal IgA levels were examined. While the stress protocol did not affect levels of the circulating cytokines in the offspring, maternal probiotic intervention down-regulated IFN-γ production (irrespective of stress conditions) and up-regulated IL-6 responses to neonatal or adult stress. Importantly however, maternal probiotic intervention enhanced immune defence capacity as indicated by increased plasma and luminal IgA. Maternal probiotic intervention was also associated with significant reductions in plasma

haptoglobin levels in all stressed and non-stressed animals to well below the baseline levels indicating enhanced loss of hemoglobin.

The third series of studies (Chapter 5) examined whether maternal probiotic intervention protected against gut microbiota and secretory state alterations induced by neonatal and/or adult stress. Neonatal and/or adult stress disrupted the normal balance of gut microbiota. Maternal probiotic intervention caused shifts in neonatal gut microflora as indicated by fostering an overgrowth of potential negative bacteria such as *E. coli*, enterococci and clostridia in stressed and non-stressed pups, resembling that of neonatally stressed pups in the vehicle subset. In adulthood maternal probiotic intervention was associated with a disruption of the normal balance of gut flora when coupled with neonatal stress, but also restoration of some gut bacterial groups to normal in stressed animals. Maternally separated animals displayed greatly decreased ileal mucin gene expression which was further decreased by exposure to adult stress. Maternal probiotic intervention decreased neonatal ileal MUC2 gene expression. In adulthood however, maternal probiotic intervention reversed the decline in mucin gene expression of stressed males.

Collectively the studies presented in the current thesis are the first to demonstrate the influence of maternal probiotic intervention on the neuroendocrine, immune and gut function in a rat model of irritable bowel syndrome. Maternal probiotic intervention exhibited mixed positive and negative effects on brain, immune and gut function, depending on age, gender and stress protocol applied. By modifying the probiotic preparations utilised (e.g., changes in the composition, dose and method of delivery) and optimising time of use, it might be possible to improve this approach to minimise the adverse outcomes. It is clear however, that maternal probiotic intervention may be a viable means to improve brain-gut outcome in ‘at risk’ neonates exposed to stress in early life and at increased risk of IBS in later life.

## Thesis Outline

A brief outline of the thesis is provided here to assist the reader. The thesis comprises six separate chapters.

**Chapter 1**

Chapter 1 provides a comprehensive review of published literature on early life stress, Irritable Bowel Syndrome and probiotics. It highlights areas of research that have not been explored in this field, and presents the research issues to be addressed in the thesis.

**Chapter 2**

Chapter 2 provides detail of the general and specific methods used in this thesis.

**Chapter 3**

Chapter 3 characterises the effect of maternal probiotic intervention on stress-induced alterations to HPA-axis activity and gut-associated neuroendocrine gene profiles.

**Chapter 4**

Chapter 4 characterises the effect of maternal probiotic intervention on stress-induced alterations to the immune system and gut-immune responses.

**Chapter 5**

Chapter 5 characterises the effect of maternal probiotic intervention on stress-induced alterations to the normal balance of gut microbiota and intestinal mucin gene expression.

**Chapter 6**

The thesis closes with Chapter 6, which includes an overall summary of the findings of this work, conclusions and recommendations for future research.

# List of abbreviations

| | |
|---|---|
| ACTH | Adrenocorticotropic hormone |
| ADHD | Attention-Deficit Hyperactivity Disorder |
| ANS | Autonomic Nervous Systems |
| APCs | Antigen-presenting Cells |
| AS | Adult Restraint Stress |
| CBG | Corticosterone-binding Globulin |
| cDNA | Complementary DNA |
| CNS | Central Nervous Systems |
| CRD | Colorectal Distension |
| CRH | Corticotropin Releasing Hormone |
| CRP | C-reactive protein |
| CT | Threshold Cycles |
| DNA | Deoxyribose Nucleic Acid |
| EMS | Emotional Motor System |
| ENS | Enteric Nervous System |
| Fbgn | Fibrinogen |
| FGIDs | Functional Gastro-intestinal Disorders |
| GF | Germ Free |
| GI | Gastrointestinal |
| GIT | Gastrointestinal Tract |
| GABA | Gamma-Aminobutyric Acid |
| GLMM | Generalised Linear Mixed Model |
| GR | Glucocorticoid receptor |
| H&E | Haemotoxylin and Eosin |
| Hp | Haptoglobin |
| HPA | Hypothalamic–Pituitary–Adrenal |
| HSD2 | 11ß-Hydroxysteroid Dehydrogenase Type 2 |
| IBS | Irritable Bowel Syndrome |
| IFN | Interferon |
| IgA | Immunoglobulin A |
| IL | Interleukin |
| IR | Immunoreactivities |
| LP | Lamina Propria |
| LPS | Lipopolysaccharide |
| MHC | Major Histocompatibility Complex |
| MNP | Myenteric Neuronal Plexus |
| MPO | Myeloperoxidase |
| MRD | Maxidam Recovery Diluents |
| mRNA | messenger RNA |
| MUC | Mucin |
| NGF | Nerve Growth Factor |
| NK | Natural Killer |
| NS | Neonatal Maternal Separation |
| NAS | Non-Adult Stress |
| NNS | Non-Neonatal Stress |
| OF | Open Field |
| PBMC | Peripheral Blood Mononuclear Cells |
| PBS | Phosphate-Buffered Saline |
| PCR | Polymerase Chain Reaction |
| PMN | Polymorphonuclear Neutrophils |
| PND | Postnatal Day |
| PVN | Para-Ventricular hypothalamic Nucleus |
| RCM | Reinforced Clostridial Medium |
| RNA | Ribonucleic Acid |
| RT-PCR | Real Time polymerase chain reaction |
| SHRP | Stress Hyporesponsive Period |
| sIL-6R | IL-6 soluble receptor |
| SNP | Submucosal Neuronal Plexus |
| TB | Toulidine Blue |
| TGF-β2 | Transforming Growth Factor β2 |
| Th | T-helper |
| TNF | Tumor Necrosis Factor |
| VIP | Vasoactive Intestinal Peptide |
| YEL | Yeast Extract Lactate |