

**Maternal Probiotic Intervention as a Prophylaxis against the
Impact of Neonatal Stress:
Implications for Irritable Bowel Syndrome**

By

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STATEMENT OF ORIGINALITY

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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
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- 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

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- 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**)

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- 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**)

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