Mechanistic target of rapamycin (mTOR) activation during ruminant mammary development and function

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in
Animal Science

At Massey University
Palmerston North
New Zealand

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2013

This thesis is dedicated to my daughter

Caitlyn Grace Sciascia

“You lift me when all else fails”

Whāia te iti kahurangi - Ki te tūohu koe, me he maunga teitei

Pursue excellence – should you stumble, let it be to a lofty mountain

ABSTRACT

This thesis examines the abundance of total and activated mechanistic target of rapamycin (mTOR) pathway components in the developing and functional ruminant mammary gland. mTOR pathway activation is stimulated by a wide range of intra- and extracellular signals, such as amino acids (AA) and hormones, making the mTOR pathway a potential candidate for the development of intervention strategies designed to increase ruminant lactation potential.

Tissues from two trials shown to improve lactation potential; dam-fetal nutrition and exogenous growth hormone (GH) administration during lactation, were used to measure changes in total and activated mTOR pathway protein abundance. Results show mammary glands of d 140 fetal lambs carried by maintenance fed dams and dairy cows administered exogenous GH, had increased abundance of total and activated mTOR and mitogen activated protein kinase (MAPK) pathway proteins. Increased abundance was associated with changes in biochemical indices. In the GH study MAPK pathway activation was stimulated by IGF-1 signaling whilst mTOR pathway activation was proposed to be mediated by AA signalling. Data from the GH study shows, L-arginine a known activator of the mTOR pathway, was the only AA reduced in both plasma and the lactating gland. Upstream factors were not identified for the phenotype observed in the dam-fetal nutrition study, but similar mechanisms were proposed.

To elucidate the potential regulation of mTOR pathway activation by L-arginine and examine the effect on milk production, *in vitro* bovine cell culture models were evaluated.

Results show that none of the models evaluated produced a lactating phenotype – a pre-requisite to accurately study the lactating gland *in vitro*.

Finally, this thesis shows L-arginine administration from d 100 to d 140 of pregnancy, in twin bearing ewes had no effect on mTOR protein abundance or activation. However, administration from d 100 to parturition improved maternal gland health.

In summary, this thesis associates improved lactation potential with increased total and activated mTOR pathway protein abundance, and the administration of L-arginine during late gestation with improved gland health. These findings provide fundamental knowledge that may lead to the development of novel technologies to increase ruminant gland performance and health.

ACKNOWLEDGEMENTS

I would like to thank my supervisors from AgResearch, Dr Sue McCoard, Dr David Pacheco and Dr Monica Senna-Salerno for all their intellectual and personal support, their dedication and guidance in seeing me over the finish line. To Dr Sue McCoard, you have become a friend; with an ear to listen, a mentor; with hands to guide and career navigator; with eyes to chart the way. To my Massey University supervisor; Prof Hugh Blair your advice has helped me immensely. ***Ehara taku toa, he taki tahi, he toa taki tini***

To my research colleagues, the molecular nutrition team (Dr Sue McCoard) Dr Danni van der Linden, Nina Wards and Francisco Sales. Thank you for your support, guidance and knowledge, we made a great team. ***Ma whero ma pango ka oti ai te mahi***

To my fellow Ph.D. students "***He waka eke noa***"

Last but not least to my whanau.

I could not have made it this far without your love, dedication and emotional support. ***Aroha nui***

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ABBREVIATIONS

4EBP1	Eukaryotic initiation factor 4E (eIF4E)-binding protein 1
4E-SE	Eukaryotic initiation factor 4E (eIF4E)-sensitivity element
AA	Amino acids
AAT	Amino acid transporter
BCAA	Branched-chain amino acids
DNA	Deoxyribonucleic acid
EAA	Essential amino acid
eIF4E	Eukaryotic initiation factor 4E
eIF4G	Eukaryotic initiation factor 4G
FAA	Free amino acid
GH	Growth hormone
IGF	Insulin-like growth factor
IGF1	Insulin-like factor 1
IGF1r	Insulin-like factor 1 receptor
IGFBP3	Insulin-like factor 1 binding protein 3
IGFBP5	Insulin-like factor 1 binding protein 5
NEAA	Non-essential amino acid
MAPK	Mitogen activated protein kinase
MKNK1	Mitogen activated protein kinase (MAPK) interacting serine/threonine kinase 1
mLST8	Mammalian lethal with SEC13 protein 8
mRNA	Messenger ribonucleic acid

mSin1	Mammalian stress-activated protein kinase (SAPK)-interacting protein 1
mTOR	Mechanistic target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
mTORC2	Mechanistic target of rapamycin complex 2
PI3K	Phosphoinositide-3-kinase
Pl	Placental lactogen
PRAS40	Proline-Rich Akt Substrate, 40 KDa
Prl	Prolactin
Raptor	Regulatory associated protein of mechanistic target of rapamycin (mTOR), complex 1.
Ras	Rat sarcoma (Protein family)
Rictor	Regulatory associated protein of mechanistic target of rapamycin (mTOR), complex 1 (RPTOR) independent companion of mTOR, complex 2
RPS6	Ribosomal protein S6
RPS6KA1	Ribosomal protein S6 kinase A1
rRNA	Ribosomal ribonucleic acid
S6K1 / S6K2	Ribosomal protein S6 kinase 1 / ribosomal protein S6 kinase 2
TSC1/TSC2	Tuberous sclerosis 1 / tuberous sclerosis 2

GENERAL INTRODUCTION

Ruminant milk production is a cornerstone of the New Zealand economy, earning in 2010 close to $15.6-billion per annum in foreign currency. In addition, the dairy industry employs locally, almost seventy-five thousand people.

The nutritional importance of milk has seen global demand rise, creating an opportunity for New Zealand dairy farmers to sell more milk, but simultaneously placing pressure on farmers to make constant productivity increases. Productivity increases that are being met through the application of novel environmental, genetic and nutritional intervention strategies. Environmental (soil maintenance, grazing intensity, water management, the introduction of new pasture species / cultivars) and genetic (breed selection, cross-breeding) advances have traditionally focused on the whole animal, whilst nutritional strategies have focused on provision of an adequate nutrient supply to the mammary gland, in an effort to increase milk production.

The ruminant mammary gland goes through very distinct developmental stages, *fetal, pre-pubertal, post-pubertal, pregnancy and lactation* with each stage susceptible to nutrient manipulation that can negatively or positively influence lactation potential. Traditionally, nutrition studies in the dairy industry have focused on plane of nutrition (high, low, and stair-step), however, results have been inconsistent, with factors such as species, age of intervention and diet composition producing conflicting results. Current research shows supplementation with specific nutrients, such as amino acids (AA), at crucial stages of

development (pregnancy, gestation, lactation), may play an important role in helping the mammary gland reach full production potential.

Amino acid uptake studies using arterio-venous differences across the lactating ruminant mammary gland show valine, leucine, arginine, lysine, and threonine are extracted in excess of milk protein outputs indicating possible roles in maintaining lactation. Arginine supplementation studies with late-pregnant Holstein cows increased milk yield, whilst the arginine-free diets retards mammary gland growth in rats. Glutamine is proposed to be limiting for milk protein synthesis due to three unique factors: uptake by the mammary gland is close to 99% of the arterial supply, high levels of glutamate are synthesised from glutamine and both glutamine, and glutamate are the most abundant AAs found in milk proteins. Arginine and glutamine belong to the arginine-family of AA (arginine, leucine and glutamine), long known to be important regulators of protein synthesis in multiple organisms. Studies show the arginine-family of AA enhance lactation potential, via possible direct or indirect stimulation of the nutrient-sensing mechanistic target of rapamycin (mTOR) pathway and reciprocal control of amino acid transporter (AAT) activity.

The mTOR pathway integrates multiple environmental signals and metabolic pathways to regulate protein synthesis, cellular proliferation and development via phosphorylation of downstream targets involved in mRNA translation and nucleocytoplasmic export, cell size, rRNA and gene transcription. The mTOR pathway may be a rate-limiting step in the cellular development and function of the ruminant mammary gland, and the ability to enhance

mTOR signalling via AA may provide a novel intervention to enhance the production of milk in ruminants.

The main objectives of this thesis were to understand the mTOR pathways role in the development and function of the ruminant mammary gland in response to external stimuli including nutrients and hormones, and to identify the potential role AAs play in stimulating mammary gland development and function through mTOR pathway signalling. The second objective was to develop a chronic *in vitro* model of bovine lactation to study the molecular mechanisms employed by AAs to regulate mTOR pathway signalling and the reciprocal control of AAT function.

Knowledge gained from this research could potentially contribute to the development of future nutritional intervention strategies designed to enhance milk protein yield, with potential economic benefits to the dairy industry.