The Role of Insulin in the Regulation of Milk Protein Synthesis in Pasture-fed Lactating Ruminants

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

Penelope Jane Back
2002

Abstract

The primary aim of this thesis was to determine the role of insulin in milk protein production in pasture-fed lactating ruminants (ewes and cows), using the hyperinsulinaemic euglycaemic clamp (HEC) technique.

Three experiments were carried out. In the first 2 experiments, the response of pasture-fed ewes and dairy cows to the HEC were established and compared to concentrate-fed ruminants (dairy cows and goats). Use of the HEC technique in pasture-fed ruminants did not result in an increase in milk protein yield or concentration. However, a reduction in feed intake along with maintenance of milk protein yield resulted in a change in efficiency of utilisation of dietary crude protein for milk protein production. This indicated that changes in blood insulin could result in changes in nutrient partitioning to maintain milk protein production.

In Experiment 3, mechanisms were examined that could maintain milk protein production despite a reduction in feed intake. The arterio-venous concentration difference technique and a leucine tracer infusion were used to measure amino acid (AA) uptake and subsequent metabolism for milk protein production under conditions imposed by the HEC. This experiment demonstrated that the HEC reduced AA supply to the mammary gland and there was a decrease in the uptake of some AA. There was no increase in mammary blood flow to compensate for this. The deficit in the ratio of AA uptake to their secretion in milk protein suggests the use of plasma free AA concentrations underestimates uptake of AA by the mammary gland and there are contributions by alternative sources such as peptide AA and erythrocytes. There was no decrease in leucine oxidation in the mammary gland, indicating that AA were not conserved for milk protein production through an alteration in this mechanism. These results support the theory that the mammary gland has the ability to respond to modified precursor supply to maintain milk protein output.

Thesis summary

The primary aim of this thesis was to determine the role of insulin in milk protein production in pasture-fed lactating ruminants (ewes and cows). To do this, the hyperinsulinaemic euglycaemic clamp (HEC) technique was utilised. This technique uses simultaneous infusions of insulin and glucose so that the role of insulin can be examined without the confounding effects of hypoglycaemia.

In the first two experiments, abomasally-cannulated ewes and rumen fistulated Jersey cows were subjected to a HEC with or without an abomasal infusion of supplemental protein (in the form of casein) in a two period cross-over design experiment.

In the experiment with lactating ewes (Chapter 3), the casein infusion resulted in significantly higher milk and milk protein yield. However, there was no increase in milk or milk protein yield with the subsequent HEC. Feed intake was significantly depressed during the HEC but as milk protein output was maintained, this resulted in an increase in the efficiency of dietary protein used for milk protein synthesis. The HEC caused a decrease in circulating concentrations of essential amino acids (EAA), particularly the branched chain AA (BCAA), leucine, isoleucine and valine.

In the experiment with lactating dairy cows (Chapter 4), there was no increase in milk or milk protein yield in response to the casein infusion. Furthermore, there was no milk protein response to the HEC in the casein-supplemented cows. However, the HEC caused milk and milk protein yield to decrease in the non-supplemented cows. As in the study with lactating ewes, feed intake was significantly reduced by the HEC, which resulted in an increase in the efficiency of dietary crude protein used for milk protein production. The HEC also reduced circulating EAA concentrations in the cows.

The data generated in both these experiments showed similar changes in variables such as changes in circulating concentrations of amino acids (AA) and energy metabolites to those observed in concentrate-fed animals where a milk protein response to the HEC alone or HEC plus supplemental protein was demonstrated. It was not clear why there was no such response in the pasture-fed animals but it may have been due to species

differences, a stage of lactation effect or the pasture-fed animals being in negative energy balance.

The third experiment used the arterio-venous (A-V) concentration difference technique and a tracer infusion of ^{13}C-leucine to examine AA uptake and subsequent metabolism for milk protein production under HEC conditions in lactating ewes. It was hypothesised that insulin (by use of the HEC without supplemental protein) stimulated an increase in AA uptake by the mammary gland, increased AA supply to the gland by increasing blood flow, and decreased AA oxidation within the gland so that AA were conserved for use in milk protein production.

As with the first two experiments, there was no increase in milk or milk protein yield under HEC conditions. The arterial supply of AA to the mammary gland was reduced but there was no change in mammary blood flow to compensate for this. Actual uptake of some EAA was reduced in insulin treated ewes. The deficit in the ratio of AA uptake to their secretion in milk protein suggests the use of plasma free AA concentrations underestimates uptake of AA by the mammary gland and there are contributions by alternative sources such as peptide AA and erthrocytes. There was no decrease in leucine oxidation in the mammary gland, indicating that AA were not conserved for milk protein production through an alteration in this mechanism. The leucine kinetics showed a tendency ($P=0.08$) for difference in irreversible loss rate but not the partitioning of leucine to the mammary gland between the control and HEC ewes. In the mammary gland, there was a lower uptake of leucine in the HEC treated ewes but no change in leucine oxidation. Although the HEC decreased total protein synthesis in the mammary gland, the ratio of leucine secreted in milk protein:gland protein synthesis was similar between the insulin treated (0.65) and control ewes (0.71), suggesting that insulin did not alter the transfer of leucine into milk protein.

These results support the theory that the mammary gland has the ability to respond to modified precursor supply to maintain milk protein output. These results are discussed in relation to work done with concentrate-fed animals.

Acknowledgements

Getting to this stage has taken a lot of help and support from numerous people, and I would like to acknowledge this. Firstly, a big thank you to my supervisors, Tricia Harris, Duncan Mackenzie, Steve Davis and Julian Lee for all their help from the animal experiments right through to numerous drafts of written work.

These kinds of experiments can be extremely demanding with long days and many samples to process. I would like to thank the many people from AgResearch and Massey who helped, particularly those who gave up their sleep to feed animals and take and process samples at all times of the night, especially Warren McNabb, Gordon Reynolds, Nicole Roy and Garry Waghorn for help with tasks as diverse as surgical preparation of the animals, development of infusion lines and the leucine kinetics study. Thanks for the brilliant technical support goes to Yvette Cottam, Bruce Sinclair, Bryan Treloar, Willy Martin and Dean Corson.

A special thank you to Arturo Luque, who put in a huge effort past the cause of friendship, and his wife Andrea, who tolerated the mad hours, fed us and coped with her husband constantly smelling of rumen fluid. And to Jason Peters (the irrepressible Mr Peters) for those interesting chats while milking and for knowing how to party!

A big thank you to my family and friends (especially the Friday night crew at the Celtic!) and other students at AgResearch who provided a good support network by listening to each others grumbles and celebrating each others successes. And to Sarah and Jason Johnston, without who this would not have been possible!

There are many people who have helped in lots of different ways that are not mentioned here. To you all thank you very much as without your help this project would not have happened!

Table of contents

Abstract i

Thesis summary ii

Acknowledgements iv

List of figures viii

List of tables ix

List of symbols and abbreviations xi

Chapter 1 Introduction. 1

1.1 Background 1

1.2 The role of insulin during lactation 3

1.3 Insulin and milk protein production 5

1.4 Amino acid supply to the mammary gland 9

1.4.1 Changes in plasma amino acid concentrations in HEC studies 9

1.4.2 Effects of insulin on splanchnic tissue and liver metabolism 10

1.4.3 Protein metabolism in skeletal muscle and how this changes during lactation 11

1.4.4 Mammary blood flow 14

1.5 Amino acid uptake by the mammary gland. 17

1.5.1 Uptake of AA by the mammary gland 17

1.5.2 Amino acid transport systems 18

1.6 The role of amino acids in milk protein synthesis. 20

1.6.1 Mammary tissue protein synthesis 20

1.6.2 The role of amino acids in milk protein synthesis. 22

1.7 Conclusion 24

1.8 Thesis objectives 25

1.9 References 26

Chapter 2 General materials and methods. 34

Chapter 3The effect of insulin on the lactation performance of pasture-fed ewes35

3.1 Abstract 35

3.2 Introduction 35

3.3 Materials and methods 37

3.3.1 Animals 37

3.3.2 Experimental Procedure 38

3.3.3 Sample collection and analyses 39

3.3.4 Statistical analysis 41

3.4 Results 42

3.5 Discussion 49

3.6 Conclusion 55

3.7 References 55

Chapter 4 The effects of insulin on the production of pasture-fed Jersey cows 60

4.1 Abstract 60

4.2 Introduction 60

4.3 Materials and methods 61

4.3.1 Animals 61

4.3.2 Experimental Procedure 63

4.3.3 Infusions 63

4.3.4 Sample collections, analyses and calculations 64

4.3.5 Statistical analysis 68

4.4 Results 69

4.5 Discussion 82

4.6 Conclusion 87

4.7 References 87

Chapter 5 Insulin and the regulation of amino acid utilisation by the lactating ewe mammary gland. 91

5.1 Abstract 91

5.2 Introduction 91

5.3 Materials and methods 92

5.3.1 Animals 92

5.3.2 Experimental Procedure *94*
5.3.3 Sample analysis and Calculations *94*
5.3.4 Leucine kinetic calculations *99*
5.3.5 Whole body calculations *99*
5.3.6 Mammary gland calculations *100*
5.3.7 Statistical analysis *101*

5.4 Results 102
5.4.1 Production data *102*
5.4.2 Amino acid and mammary blood flow data *106*
5.4.3 Leucine kinetic results *114*

5.5 Discussion 116
5.5.1 Milk production data *117*
5.5.2 Blood flow *119*
5.5.3 Amino acid utilisation by the mammary gland *121*
5.5.4 Leucine kinetics *123*

5.6 Conclusion 128

5.7 References 128

Chapter 6 General discussion. 134

6.1 Energy balance and effect on feed intake 136

6.2 Genetic component 136

6.3 Ewe as a model / species difference 137

6.4 Future work 138

6.5 References 139

Chapter 7 Appendices 141

7.1 Appendix A 141
7.1.1 Sample Preparation *141*
7.1.2 Sample Chromatography *141*
7.1.3 Lactose Concentration Calculation *141*

7.2 Appendix B 142

7.3 Appendix C 144

7.4 Appendix D 145

List of figures

Fig. 3-1 Plot of group means generated from raw data to graphically illustrate the type of changes that occurred over 2 periods of insulin infusion over the 28 day experiment. 44
Fig. 3-2 Shows the response of selected essential amino acids to the hyperinsulinaemic euglycaemic clamp (HEC), where concentrations were measured on day 8 of infusion period (day 4 casein infusion), day 9 (day 1 HEC) and day 12 (day 4 HEC). 48
Fig. 5-1 The relationship between blood flow measured with the transit time flow probes (ml/min) and amino acid A-V differences using the Fick Principle. 111
Fig. 5-2 Schematic model showing leucine fluxes in ewe mammary gland during HEC. 126

List of tables

Table 1-1 Comparison of changes in milk protein yield and composition in hyperinsulinaemic euglycaemic clamp studies. 8
Table 1-2 Possible amino acid transport systems in bovine mammary tissue and (% extraction) derived from A-V studies. 19
Table 2-1 Locations for details of materials and methods used in this thesis. 34
Table 3-1 Comparison of feed intakes and energy balance on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 43
Table 3-2 Comparison of milk yield and milk component yields on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 45
Table 3-3 Comparison of concentrations of circulating amino acid in plasma on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 46
Table 3-4 Comparison of circulating concentrations of insulin and energy metabolites on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 49
Table 4-1 Plasma insulin and blood glucose concentrations on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 70
Table 4-2 Comparison of feed intakes and energy balance on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 71
Table 4-3 Comparison of actual and predicted feed intakes and milk yield on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp during both periods of infusion. 72
Table 4-4 Comparison of milk yield, yield and concentration of milk components on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 73
Table 4-5 Circulating concentrations of amino acids in plasma on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 76
Table 4-6 Comparison of plasma metabolite concentrations on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 78
Table 4-7 Comparison of milk fatty acids concentrations on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 79
Table 4-8 Comparison of milk fatty acids yields on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 80
Table 4-9 Comparison of circulating concentrations of IGF-1 and cortisol in plasma on day 4 of the infusion period and day 4 of the hyperinsulinaemic euglycaemic clamp. 81
Table 4-10 Concentrations of minerals in milk on day 4 of the casein infusion and day 4 of the hyperinsulinaemic euglycaemic clamp. 82
Table 5-1 Comparison of intakes of dry matter, crude protein, energy and energy balance and dietary crude protein utilisation on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 103
Table 5-2 Comparison of milk yield, yield and concentration of milk components and individual milk proteins on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 104
Table 5-3 Arterial concentrations, A-V differences and mammary gland extraction efficiencies of acetate, triacylglycerols and β-hydroxybutyrate during day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 105

Table 5-4 Comparison of plasma arterial concentrations of amino acids on day 4 of hyperinsulinaemic euglycaemic clamp between treated and control ewes. 107
Table 5-5 Comparison of plasma amino acid A-V differences across the mammary gland on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 108
Table 5-6 Comparison of mammary extraction efficiency by the mammary gland of plasma amino acids on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 109
Table 5-7 Comparison of blood flow estimates by different methods with 6 ewes and by methionine concentration with 12 ewes on day 4 of the hyperinsulinaemic euglycaemic clamp. 110
Table 5-8 Comparison of net uptakes of plasma amino acids by the mammary gland on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 112
Table 5-9 Ratio of AA uptake:AA secreted in milk protein during day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 113
Table 5-10 Comparison of mammary glucose utilisation on day 4 of the hyperinsulinaemic euglycaemic clamp. 114
Table 5-11 Comparison of isotopic enrichments and whole body leucine kinetics on day 4 of the hyperinsulinaemic euglycaemic clamp between treated and control ewes. 115
Table 5-12 Mammary gland leucine kinetics in insulin treated and control ewes on day four of the hyperinsulinaemic euglycaemic clamp. 116

List of symbols and abbreviations

AA	Amino acid
ANOVA	Analysis of variance
BCAA	Branch chain amino acids
BF	Blood flow
BW	Body weight
C_A	Arterial concentration of $C0_2$
CO_2	Carbon dioxide
CP	Crude protein
C_V	Venous concentration of $C0_2$
CV	Coefficient of variation
d	Day
DM	Dry matter
DMI	Dry matter intake
EAA	Essential amino acid
$E_{C,A}$	Isotopic enrichment of arterial CO_2
$E_{C,V}$	Isotopic enrichment of venous CO_2
$E_{K,A}$	Isotopic enrichment of arterial KIC
$E_{K,V}$	Isotopic enrichment of venous KIC
$E_{L,A}$	Isotopic enrichment of arterial leucine
$E_{L,V}$	Isotopic enrichment of venous leucine
Ey	Isotopic enrichment of either venous leucine or KIC as the precursor pool
g	Gram
H_2	Hydrogen
Ha	Hectare
HCl	Hydrochloric acid
$HClO_4$	Perchloric acid
He	Helium
HEC	Hyperinsulinaemic euglycaemic clamp
HNO_3	Nitric acid
HPLC	High performance liquid chromatography

h	Hour
ICP	Inductive coupled plasma emission spectometry
IE	Isotopic enrichment
IGF-1	Insulin-like growth factor-1
ILR	Irreversible loss rate
ILR_{ALEU}	Irreversible loss rate of arterial leucine
K_A	Arterial concentration of KIC
kg	Kilogram
KIC	Keto isocaproate acid
KOH	Potassium hydroxide
K_V	Venous concentration of KIC
L_A	Arterial concentration of leucine
Leu	Leucine
LO	Leucine oxidation
LSMeans	Least squares means
L_V	Venous concentration of leucine
MBF	Mammary blood flow
mg	Milligram
min	Minute
MJ ME	Mega joules metabolisable energy
ml	Millilitre
mm	Millimetre
mM	Millimole
MPE	Moles percent excess
MPE_{ALEU}	Mole percent excess of arterial leucine
MS	mass spectometer
N	Nitrogen
Na_2EDTA	Disodium ethylenediaminetetraacetate
NEAA	Non essential amino acids
NEFA	Non esterfied fatty acids
ng	Nanogram
NIRS	Near infrared reflectance spectroscopy
NITS	Near infrared transmittance spectroscopy
NO_2	Nitrous oxide

NPN	Non protein nitrogen
NRC	National research council
O_2	Oxygen
P	Probability
pg	Picogram
RIA	Radioimmunoassay
RPM	Revolutions per minute
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of the mean
SSA	Sulphosalicylic acid
Sy	Precursor pool for leucine or KIC
t	Time
TCA	Trichloroacetic acid
µl	Microlitre
UV	Ultra violet
VFA	Volatile fatty acid