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**Effect of mechanical stress on the
integrity, signalling mechanisms and
function of bovine mammary epithelial
cells**

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Abstract

Mammary gland engorgement due to milk accumulation in late lactation leads to changes in cell morphology and has been recognised as a potential key initiator of involution and remodelling of the mammary gland. The physical distension of mammary epithelial cells (MEC), due to udder filling, is likely to result in mechanical tension on cell-cell and cell-matrix interactions. Cell-cell and cell-matrix junctions provide tissue integrity, promote cell polarity, guarantee sufficient communication between cells to ensure synchronised milk secretion and support cell survival. Their disruption may be one of the early initiators of the mammary gland remodelling process. As a consequence, the primary goal of this study was to determine the potential effects of MEC stretch on changes in cell sensing within the mechanical micro-environment in the initiation of bovine MEC involution. During this investigation, particular emphasis was put on three potential mechanosensors: tight junctions (TJ), focal adhesions (FA) and primary cilia (PC), and their regulation in the early stages of involution using *in vivo* and *in vitro* experimental approaches. Static, biaxial *in vitro* cell stretch and acute physical distension *in vivo* resulted in changes in TJ protein expression levels implying a potential disruption of cell-cell communication as well as communication with the cell's cytoskeleton. Furthermore, down-regulation of Akt and pAkt following different periods of mechanical strain applied *in vitro* and decreased levels of pAkt following acute physical distension *in vivo* indicated a disruption of β 1-integrin-FAK survival signalling through the PI3K-Akt pathway downstream of FA interactions. Increased numbers of ciliated MEC following extended periods of non-milking indicated a dedifferentiation of MEC.

Furthermore, increased levels of STAT6 transcription (part of PC signalling following mechanical stimulation) factor indicates the initiation of macrophage accumulation and promotion of tissue remodelling of the bovine mammary gland. In conclusion, this study supports the hypothesis that local factors play an important role during bovine mammary gland involution and that mechanical stimulation may play a part in the initiation of this process.

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Contents

Abstract.....	i
Acknowledgements.....	iii
List of Figures	ix
List of Tables	xiii
List of Abbreviations.....	xiv
Chapter 1 General Introduction	1
1.1 Dairy industry	1
1.2 Mammary gland	3
1.2.1 Structure of the mammary gland	3
1.2.2 Secretory cells of the lactating mammary gland	5
1.2.3 Extracellular matrix.....	13
1.3 Lactation cycle	18
1.3.1 Lactogenesis	18
1.3.2 Galactopoeisis.....	20
1.3.3 Involution	23
1.4 Mechanobiology.....	27
1.4.1 The role of integrins, tight junctions, and primary cilia as mechanosensors	30
1.4.2 Mechanotransduction in <i>in vitro</i> studies	37
1.5 Objective of this study	44
Chapter 2 Introduction	47
2.1 Primary cilium	49
2.1.1 Mechanotransduction at the primary cilium	51
2.1.2 STAT6/p100 pathway.....	52
2.1.3 Objective	53
2.2 Materials and Methods.....	54

2.2.1 Animal experiment	54
2.2.2 Tissue collection	54
2.2.3 Fluorescent immunohistochemistry	55
2.2.4 RNA preparation	58
2.2.5 Protein preparation	63
2.2.6 Statistical analysis	66
2.3 Results	68
2.3.1 Histological analysis	68
2.3.2 Confocal imaging of primary cilia in mammary tissue	73
2.3.3 Comparison of manual and automatic nuclei count	78
2.3.4 Primary cilia analysis	80
2.3.5 Analysis of qRT-PCR.....	84
2.3.6 Western blot analysis of (p)STAT6 protein expression	88
2.4 Discussion	92
Chapter 3 Introduction	97
3.1 Mechanotransduction	98
3.1.1 Mechanotransduction in <i>in vitro</i> studies.....	99
3.1.2 Objective	101
3.2 Materials and Methods	102
3.2.1 Cell stretch device	102
3.2.2 Primary cell extraction	107
3.2.3 Cell culture.....	108
3.2.4 Immunocytochemistry.....	109
3.2.6 Cell stretch experiments	111
3.2.7 Sample extraction	112
3.2.8 Protein preparation	117
3.2.9 Statistical analysis	119

3.3 Results	121
3.3.1 Histological analysis	121
3.3.2 Assessment of different membrane coatings.....	123
3.3.3 qRT-PCR results after <i>in vitro</i> stretch experiments	125
3.3.4 Analysis of levels of protein expression after <i>in vitro</i> stretch experiments.....	129
3.4 Discussion.....	134
Chapter 4 Introduction	139
4.1.1 Local control of milk production.....	141
4.1.1.1 Alveolar distension	142
4.1.1.2 Mechanotransduction	143
4.1.2 Objective	145
4.2 Materials and Methods.....	146
4.2.1 Animal experiment.....	146
4.2.2 Histological analysis	147
4.2.3 RNA preparation.....	148
4.2.4 Protein preparation.....	154
4.2.5 Statistical analysis	157
4.3 Results	159
4.3.1 Histological analysis	159
4.3.2 Milk composition data	162
4.3.3 qRT-PCR results after infusion experiment	167
4.3.4 Analysis of levels of protein expression after infusion experiment	171
4.4 Discussion.....	176
Chapter 5 General Discussion and Conclusions	181
Chapter 6 References	181
Appendix I: List of publications arising from this project	205

7.1 Conference abstracts 205

 7.1.1 Poster presentations 205

7.2 Oral presentations 206

List of Figures

Figure 1.1 Schematic of an alveolus.....	5
Figure 1.2 Schematic diagram of epithelial cells and the principal types of cell junctions that connect them.....	7
Figure 1.3 Schematic representation of the tight junction.....	9
Figure 1.4 Schematic of structure of a primary cilium.....	12
Figure 1.5 Schematic of the connective tissue underlying an epithelium.....	14
Figure 1.6 Schematic representation of the basic structure of the integrin	31
Figure 1.7 Schematic of common mechanical forces acting on cells in vivo.	32
Figure 1.8 Response pathway of primary cilia to fluid-flow-induced bending.....	35
Figure 2.1 Hematoxylin and eosin-stained sections of mammary alveolar tissue from animals following extended periods of non-milking	72
Figure 2.2 Representative composite images of primary cilia in lactating alveolar tissue sections.....	75
Figure 2.3 Representative composite images of primary cilia in alveolar tissue sections from the 7-d nm (non-milking) group.	76
Figure 2.4 Representative composite images of primary cilia in alveolar tissue sections from the 28-d nm (non-milking) group.	77
Figure 2.5 Comparison of manual and automatic nuclei count.....	79
Figure 2.6 Average of total and luminal primary cilia within each experimental group.....	80
Figure 2.7 Percentage of cilia detected per treatment group (\pm sem).....	81
Figure 2.8 Representative composite micrographs of variations in cilium morphogolgy.....	82
Figure 2.9 Average number of different primary cilia morphologies within each experimental group.	84
Figure 2.10 Differences in gene expression levels of α s1-casein, α -lactalbumin and κ -casein following extended periods of non-milking.....	86
Figure 2.11 Changes in gene expression levels of lactoferrin and stat6 following extended periods of non-milking.	87

Figure 2.12 Representative image of a coomassie blue stained gel loaded with protein samples extracted from bovine mammary glands following extended periods of non-milking.	88
Figure 2.13 Representative image of western blot after stat6-antibody testing.	89
Figure 2.14 Representative image of western blot to determine patterns of stat6 protein expression following extended periods of non-milking.....	91
Figure 2.15 Densitometric analysis of western blots to determine changes in stat6 protein expression in the mammary gland following extended periods of non-milking.	91
Figure 3.1 A custom-made device to stretch mecs <i>in vitro</i>	103
Figure 3.2 Custom-made cell stretch device fully assembled under the microscope.....	103
Figure 3.3 Schematic of the principal function of a custom-made device designed to stretch mecs <i>in vitro</i>	105
Figure 3.4 The relationship between the indentation depth (mm) of the cell-stretch device and the maximum % change in membrane surface area (δ msa).....	107
Figure 3.5 Representative images of icc staining to confirm epithelial phenotype in cell culture, including positive and negative control.	122
Figure 3.6 Representative images of primary cells grown for 9 days on different types of membrane precoating.	124
Figure 3.7 Changes in α s1-casein gene expression levels following different periods of static stretch applied <i>in vitro</i>	125
Figure 3.8 Changes in tj gene expression levels following different periods of static stretch applied <i>in vitro</i>	126
Figure 3.9 Changes in β 1-integrin gene expression levels following different periods of static stretch applied in vitro.	127
Figure 3.10 Changes in bax and bcl-2 gene expression levels following different periods of static stretch applied in vitro.	128
Figure 3.11 representative image of a coomassie blue stained gel loaded with protein samples extracted from bovine primary cell culture experiments following extended periods of <i>in vitro</i> stretch.	129

Figure 3.12 Representative image of western blot to determine patterns of tj protein expression following extended periods of <i>in vitro</i> cell stretch.....	130
Figure 3.13 Densitometric analyses of western blots to determine changes in tj protein expression levels following <i>in vitro</i> cell stretch.	131
Figure 3.14 Representative image of western blot to determine patterns of (p)akt protein expression following extended periods of <i>in vitro</i> cell stretch.....	132
Figure 3.15 Densitometric analyses of western blots to determine changes in (p)akt protein expression levels following <i>in vitro</i> cell stretch.	133
Figure 4.1 Morphological differences between the control and infused treatment group.	161
Figure 4.2 Lactation grades after assessment of histological features to compare morphological differences between the control and treatment group.	162
Figure 4.3 Milk fat content determined in milk samples collected from each hind quarter (control and treatment) before and after the infusion experiment.	163
Figure 4.4 Total milk protein content determined in milk samples collected from each hind quarter (control and treatment) before and after the infusion experiment.....	164
Figure 4.5 Somatic cell count determined in milk samples collected from each hind quarter (control and treatment) before and after the infusion experiment.....	165
Figure 4.6 Lactose content determined in milk samples collected from each hind quarter (control and treatment) before and after the infusion experiment.....	166
Figure 4.7 Total milk solids content determined in milk samples collected from each hind quarter (control and treatment) before and after the infusion experiment.....	167
Figure 4.8 Changes in gene expression levels of α s1-casein, α -lactalbumin and lactoferrin following the infusion of one hind quarter compared with non-infused controls.	168

Figure 4.9 Changes in gene expression levels of tj proteins, zo-1, occludin, and claudin, following the infusion of one hind quarter compared with non-infused controls.	169
Figure 4.10 Changes in gene expression levels of β 1-integrin, bcl-2 and bax, following the infusion of one hind quarter compared with non-infused controls.....	170
Figure 4.11 Representative image of ponceau s-stained nitrocellulose membrane after protein transfer.....	171
Figure 4.12 Representative images of western blots to determine changes in tj protein expression following the infusion experiment	172
Figure 4.13 Densitometric analysis of western blots to determine changes in tj proteins expressions following the infusion experiment.	173
Figure 4.14 Representative images of western blots to determine patterns of (P)AKT protein expression following the infusion experiment..	174
Figure 4.15 Densitometric analysis of western blots to determine changes in (P)AKT protein expressions following the infusion experiment.....	175

List of Tables

Table 1.1 Summary of cell responses to mechanical stimuli in various stretch experiments, cpm: cycles per minute (1 cycle = stretch + relaxation).....	40
Table 1.2 cont. Summary of cell responses to mechanical stimuli in various stretch experiments, cpm: cycles per minute (1 cycle = stretch + relaxation).....	41
Table 1.3 cont. Summary of cell responses to mechanical stimuli in various stretch experiments, cpm: cycles per minute (1 cycle = stretch + relaxation).....	42
Table 1.4 cont. Summary of cell responses to mechanical stimuli in various stretch experiments, cpm: cycles per minute (1 cycle = stretch + relaxation).....	43
Table 2.1 Sequences of PCR primers (forward and reverse), primer position, PCR product sizes and individual experimental conditions of bovine nucleic acid sequences used for investigating gene expression by real-time RT-PCR	61
Table 2.2 Information on antibodies used for western blot analysis, including species of origin, molecular weight, supplier, dilution and gel percentage for used sds page gel electrophoresis.....	66
Table 3.1 Sequences of PCR primers (forward and reverse), primer position, PCR product sizes and individual experimental conditions of bovine nucleic acid sequences used for investigating gene expression by real-time RT-PCR	115
Table 3.2 Information on antibodies used for western blot analysis, including species of origin, molecular weight, supplier, dilution and gel percentage for used SDS page gel electrophoresis.....	119
Table 4.1 Sequences of PCR primers (forward & reverse), primer position, PCR product sizes & individual experimental conditions of bovine nucleic acid sequences used for investigating gene expression by quantitative real-time PCR.....	151
Table 4.2 cont. Sequences of PCR primers (forward & reverse), primer position, PCR product sizes & individual experimental conditions of bovine nucleic acid sequences used for investigating gene expression by quantitative real-time PCR.....	152

List of Abbreviations

1X	Once daily milking
2X	Twice daily milking
3X	Thrice daily milking
BM	Basement membrane
CTK18	Cytokeratin 18
DIM	Days in milk
ECM	Extracellular matrix
EGF	Epidermal growth factor
FA	Focal adhesion
FAC	Focal adhesion complex
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IFT	Intraflagellar transport
JAM	Junctional adhesion molecule
MEC	Mammary epithelial cell
NFMP	Non-fat milk powder
PC	Primary cilium
PC-1	Polycystin-1
PC-2	Polycystin-2
PRL	Prolactin
PRLR	Prolactin receptor
qRT-PCR	Quantitative real time-polymerase chain reaction
RIN	RNA integrity number
RT	Room temperature
SCC	Somatic cell count
SMA	Smooth muscle actin
TJ	Tight junction
TRP	Transient receptor potential
ZO-1/2/3	Zonula occludens 1/2/3