Intra-mammary molecular mechanisms involved in the response to changes in milking frequency

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Abstract

In dairy cows, short-term changes of milking frequency (MF) in early lactation have been shown to produce an immediate and a long-term effect on milk yield (MY). The effect is controlled locally within the mammary gland by as yet unknown factors. To investigate the intra-mammary molecular mechanisms that are involved in the MY response to MF, a unilateral milking frequency (UMF) experiment was conducted with udder halves of 17 multiparous pasture-fed dairy cows milked either four times a day (4x) or once a day (1x) for fourteen days from 5 ± 2 days in milk (DIM). Mean udder-half MY during the treatment period was higher from the 4x compared to 1x-udder halves and once returned to twice a day milking, continued to be higher until 200 DIM. Mammary biopsies were obtained on day fourteen of treatment from both udder halves of ten cows. Proliferation of mammary cells was higher in 4x-udder halves compared to 1x, whereas no difference in apoptosis levels was detected. Abundance of major milk protein gene mRNA was higher in tissue samples from 4x-udder halves compared with 1x. The effects of changes of MF in early lactation on the prolactin (PRL) and insulin-like growth factor I (IGF-I) pathways to determine their role in the MY response to MF. The activation of signal transducer and activator of transcription (STAT) 5 was measured as an indication of PRL signalling, which was higher following 4x-milked mammary tissue samples compared to 1x-milked, and correlated highly with milk protein gene mRNA abundance. Activation of STAT5 also correlated with the protein abundance of the extracellular matrix (ECM) interacting protein β1-integrin, which suggest a

link between PRL/STAT5 and ECM/β1-integrin signalling. The mRNA abundance of IGF binding protein (IGFBP)3 and IGFBP5 were lower in 4x-milked mammary tissue samples relative to 1x-milked. Both IGFBP3 and IGFBP5 are thought to inhibit IGF-I, so the decrease in their mRNA abundance may serve to stimulate the IGF-I signal in the 4x-milked mammary gland. However, two cellular pathways downstream of IGF-I (phosphoinositide 3-kinase (PI3K)/Akt and extracellular-signal-regulated kinase (ERK)1/2) were not positively affected by 4x milking. The activation of PI3K/Akt pathway was lower in 4x-milked mammary tissue samples relative to 1x-milked, and the activation of the ERK1/2 was unaffected by MF. Overall, the results obtained in this thesis have increased the understanding of the changes in intra-mammary molecular mechanisms in response to differing MF.

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Publications arising from this thesis

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Abbreviations

1x	once a day milking
2x	twice a day milking
3x	three times a day milking
4x	four times a day milking
B2M	β-2 microglobulin
BAX	Bcl-2-associated X protein
BCL-XL	B-cell lymphoma-extra-large
BSA	bovine serum albumin
C_t	threshold cross-over value
CIS	cytokine inducible SH2 protein
CP	crude protein
CSN1S1	α_{S1}-casein
CSN2	β-casein
DIM	days in milk
DM	dry matter
ECM	extracellular matrix
ERK	extracellular-signal-regulated kinase
FAK	focal adhesion kinase
GRB2	growth factor receptor-bound protein 2
GH	growth hormone

HRP	horseradish peroxidase
IGF1	insulin-like growth factor I gene
IGF1R	insulin-like growth factor type I receptor gene
IGFBP	IGF binding protein
IGF-I	insulin-like growth factor I
IGFIR	insulin-like growth factor type I receptor
IRS	insulin receptor substrate
JAK	janus kinase
LALBA	α-lactalbumin
LGB	β-lactoglobulin
LTF	lactoferrin
LIF	leukaemia inhibitory factor
Lu	lumen
ME	metabolisable energy
MEC	mammary epithelial cell
MF	milking frequency
MY	milk yield
NS	not significant
PI3K	phosphoinositide 3-kinase
PRL	prolactin
PRLR	prolactin receptor

Rac1	Ras-related C3 botulinum toxin substrate 1
RIN	RNA integrity number
SCC	somatic cell count
SED	standard error of the difference
SEM	standard error of the mean
SHC	src/collagen homology protein
SOCS	suppressor of cytokine signalling
STAT	signal transducer and activator of transcription
TBS	tris buffered saline
TBST	tris buffered saline containing 1% tween 20
UBB	ubiquitin B
UMF	unilateral milking frequency