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The effects of milking frequency on insulin-like growth factor I signaling within the mammary gland of dairy cows

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ABSTRACT

In dairy cows, short-term changes in milking frequency (MF) in early lactation have been shown to produce both an immediate and a long-term effect on milk yield. The effect of MF on milk yield is controlled locally within mammary glands and could be a function of changes in either number or activity of secretory mammary epithelial cells (MEC). Insulin-like growth factor I (IGF-I) signaling is one candidate factor that could mediate these effects, as it can be controlled locally within mammary glands. Both MEC number and activity can be affected by IGF-I signaling by activating the phosphoinositide 3-kinase (PI3K)/Akt and extracellular-signal-regulated kinase (ERK)1/2 pathways. To investigate the relationship between MF and IGF-I signaling, udder halves of 17 dairy cows were milked either 4 times a day (4×) or once a day (1×) for 14 d in early lactation. On d 14, between 3 and 5 h following milking, mammary biopsies were obtained from 10 cows from both udder halves, and changes in the expression of genes associated with IGF-I signaling and the activation of the PI3K/Akt and ERK1/2 pathways were measured. The mRNA abundance of IGF type I receptor, IGF binding protein (*IGFBP*)*-3*, and *IGFBP-5* were lower following 4× milking relative to 1× milking. However, the mRNA abundance of *IGF-I* was not affected by MF. Both IGFBP3 and IGFBP5 are thought to inhibit IGF-I; therefore, decreases in their mRNA abundance may serve to stimulate the IGF-I signal in the 4×-milked mammary gland. The activation of PI3K/Akt pathway was lower in response to 4× milking relative to 1×, and the activation of the ERK1/2 was unaffected by MF, suggesting that they do not mediate the effects of MF.

Key words: milking frequency, mammary, milk synthesis, cell signaling

INTRODUCTION

In early lactation, short-term changes of milking frequency (**MF**) have an immediate effect on milk yield (**MY**), as well as a long-term MY effect after normal milking is resumed (Bar-Peled et al., 1995; Rémond et al., 1999). The positive long-term carry-over effect on MY of increased MF can be achieved with a 2- to 3-wk treatment of 4 times a day (**4×**) milking compared with twice a day (**2×**) milking alone (Hale et al., 2003; Wall and McFadden, 2007). The negative carryover effect on MY in response to decreased MF has also been demonstrated following once-a-day (**1×**) milking, although in this case, a 1× treatment of at least 6 wk is required (Rémond et al., 1999).

Unilateral MF (**UMF**) experiments, in which udder halves are milked at different frequencies independently of one another, have demonstrated that the effects of MF on MY are predominantly controlled by intramammary factors (Stelwagen and Knight, 1997; Wall and McFadden, 2007; Murney et al., 2015a). Milk yield is ultimately affected by a combination of the number of secretory mammary epithelial cells (**MEC**) and their level of activity; therefore, factors and signaling pathways that modulate these parameters are the most plausible candidate mechanisms driving the response to changes in MF. Comparison of 4× milking with 1× milking by UMF in early lactation demonstrated an increase in both proliferation and the mRNA abundance of the major milk protein genes in response to the higher MF (Murney et al., 2015a). Furthermore, analysis of mammary tissue from these treatments showed that signaling proteins involved in prolactin and extracellular matrix signaling pathways seem to be affected by MF (Murney et al., 2015b). However, these effects do not provide a full mechanistic description of how the MF effects are mediated in the mammary gland. Thus, further candidate pathways and factors should be investigated.

Insulin-like growth factor-I (IGF-I) is a candidate factor that may be involved in the effects of MF. This endocrine hormone is synthesized in the liver in response to growth hormone and it circulates in the

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bloodstream at a relatively high concentration. In addition, IGF-I can be locally produced within several different tissues, including mammary glands, thereby acting as an autocrine hormone (Glimm et al., 1992). This locally produced IGF-I is thought to be as important to mammary gland development as systemic IGF-I (Akers et al., 2000).

The majority of IGF-I found in circulation is bound with high affinity to one or more of the 6 known IGF binding proteins (**IGFBP 1–6**), which modulate the bioavailability of IGF-I to target tissues (Flint et al., 2008). In mammary glands, locally synthesized IGFBP3 and IGFBP5 are thought to be important for controlling IGF-I function (Flint et al., 2008). The upregulation of IGFBP5 has been demonstrated in rodent mammary glands at the onset of involution and is thought to inhibit the cell survival signal of IGF-I (Tonner et al., 1997; Flint et al., 2005). The effects of IGF-I on cellular function can also be moderated by changes in the levels of the IGF type I receptor (**IGFIR**), which is the primary signaling receptor for IGF-I (Baumrucker and Erondu, 2000).

Insulin-like growth factor-I is a potent mammary mitogen and has been shown both to have proliferative properties in bovine MEC in vitro (McGrath et al., 1991) and to protect against apoptosis in mouse mammary glands during involution (Neuenschwander et al., 1996). Furthermore, IGF-I has been shown to increase milk secretion in goats when administered via close arterial injection (Prosser et al., 1990). Interestingly, this effect is masked by a preceding treatment of increased MF, suggesting some link between the effect of IGF-I and the effect of MF (Prosser and Davis, 1992).

Previous studies have reported changes in components of the IGF-I signaling pathway within mammary glands in response to MF (Bernier-Dodier et al., 2010; Littlejohn et al., 2010; Wall and McFadden, 2010; Boutinaud et al., 2013). The abundance of *IGF-I* and *IGFIR* mRNA increased in mammary tissue that was subjected to 3-times-a-day (**3**×) milking in mid-lactation compared with 1× milking, whereas *IGFBP5* mRNA was not affected (Bernier-Dodier et al., 2010). In contrast, microarray analysis of genes modulated in response to 1× milking revealed an increase of *IGFBP5* mRNA abundance in 1×-milked udder halves compared with 2× (Littlejohn et al., 2010; Boutinaud et al., 2013). In another study comparing 4× and 2× milking in early lactation, no MF effect was observed on *IGF-I* and *IGFIR* mRNA abundance, but lower amounts of *IGFBP3* mRNA were observed in 4×-milked mammary tissues compared with 2× (Wall and McFadden, 2010). This response of *IGFBP3* mRNA to MF was only detected when the tissue was collected following a 4× milking, whereas samples taken when both udder halves were milked showed no difference in *IGFBP3* mRNA abundance (Wall and McFadden, 2010). Overall, the differing findings of these studies only serve to illustrate the complex and dynamic nature of IGF-I signaling in bovine mammary glands, which is not fully understood.

Two intracellular pathways are thought to be stimulated by IGF-I: the phosphoinositide 3-kinase (**PI3K**)/Akt and extracellular-signal-regulated kinase (**ERK**)1/2 pathways (Peruzzi et al., 1999). The serine/threonine kinase Akt is an important intracellular node in cell signaling, which is activated by IGF-I as well as other growth factors, cytokines, and the extracellular matrix. Activation of Akt is known to protect against apoptosis (Kennedy et al., 1997) and overexpression of activated Akt within the mammary glands of transgenic mice can delay the onset of involution (Hutchinson et al., 2001). The ERK1/2 pathway is also stimulated by IGF-I as well as by other mitogenic stimuli. Activation of the ERK1/2 pathway is involved in a diverse range of cellular processes that control functions such as cell cycle progression, cell proliferation, cell division, and cell differentiation. The activation of ERK1/2 is essential for appropriate development of mammary glands controlling MEC proliferation (Whyte et al., 2009). These signaling intermediates thus represent likely downstream candidate mediators of the MF response in mammary glands.

We hypothesized that MF modulates the IGF-I signaling pathway within mammary glands by altering the expression of locally produced IGF-I, IGFBP, and IGFIR. This would in turn lead to activation of the PI3K/Akt or ERK1/2 pathways, or both, which results in modulation of the rate of MEC turnover by altering levels of apoptosis and proliferation. To test this hypothesis, we measured several key components of the IGF-I signaling pathway in a 4×/1× UMF model.

MATERIALS AND METHODS

Animals and Treatments

All animal manipulations were conducted in compliance with the rules and guidelines of the Ruakura Animal Ethics Committee. Animal management and treatments have been described in detail previously (Murney et al., 2015a). Briefly, half udders from 17 Holstein-Friesian and Holstein-Friesian × Jersey dairy cows in the first week of lactation (5 ± 2 DIM) were randomly assigned to MF treatment, either 4× or 1× (4× in one udder half at 0500, 1100, 1700, and 2300 h, and 1× in the other udder half at 1100 h) for 14 d. The pretreatment MY of the udder halves were 8.4 ± 0.5 and 8.5 ± 0.5 kg/d for 4× and 1×, respectively. By