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Gene expression in liver and adipose tissue is altered during and after temporary changes to postpartum milking frequency

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ABSTRACT

Short-term changes to milking frequency can alter the metabolic status of dairy cows depending on the duration, magnitude, and stage of lactation at which the milking frequency changes occur. Additionally, effects of altered milking frequency that are subsequent to cows returning to a normal twice-daily (2×) milking regimen are not well established. This study tested the hypothesis that plasma concentrations of key hormones and metabolites and transcription of genes involved in the somatotropic axis and lipid metabolism would be altered in liver and subcutaneous adipose tissue from cows milked with different frequencies. Multiparous Holstein-Friesian dairy cows were allocated to 2× milking for the whole lactation, or once-(1×) or 3 times-(3×) daily milking for 3 or 6 wk, immediately postpartum, and then 2× milking for the remainder of the lactation. Liver and subcutaneous fat were biopsied at wk 1 (liver only), 3, 6, and 9 postpartum, and transcription of genes involved in the somatotropic axis and lipid metabolism were measured. At wk 3, cows milked 3× had lower hepatic expression of growth hormone receptor (*GHR1A*) compared with cows milked 2× or 1×, and lower *IGF1* expression compared with cows milked 1×, indicating greater uncoupling of the somatotropic axis. At wk 6, reduced transcription of total *GHR* and *GHR1B* occurred in the adipose tissue of cows milked 3×. Cows milked 1× had greater transcription in adipose tissue of lipogenesis genes at wk 3 and 6, and lipolysis genes at wk 6, compared with cows milked 2×, indicating a period of increased fatty acid storage, followed by increased fatty acid reesterification. At wk 9, cows previously milked 3× for 6 wk maintained lower transcription of genes involved in lipogenesis, lipolysis, and ketolysis in adipose tissue compared with cows milked 2×, indicating that the effects of 3× milking persist for at least 3 wk after switching to 2× milking. Results indicate that alterations to milking frequency affect the transcription of genes involved in lipid mobilization and storage, enabling the animal to manage the energy demands associated with the change in milk production. Some of these gene transcription changes were maintained in cows previously milked 3×, indicating that the adipose tissue gene expression changes were still required even after 3 wk of the less-demanding 2× milking regimen.
Key words: energy balance, somatotropic axis, early lactation, lipid metabolism

INTRODUCTION

During early lactation, homeorhetic changes ensure that nutrients are partitioned toward milk production at the expense of tissue reserves (Bauman and Currie, 1980). These changes include a period of insulin resistance (Bell and Bauman, 1997; Vernon and Pond, 1997) and the uncoupling of the somatotropic axis, which is reflected by decreased growth hormone (**GH**) receptor (**GHR**) expression in the liver (Kobayashi et al., 1999). The reduced GHR expression limits the hepatic production of IGF-1 (Lucy et al., 2009), and decreases the negative feedback of IGF-1 on GH production (Rhoads et al., 2004). The insulin resistance, high plasma GH, and low IGF-1 concentrations act in coordination to minimize glucose uptake into muscle and adipose tissue (Chagas et al., 2009; Lucy et al., 2009), and enhance lipolysis in adipose tissue (McNamara and Hillers, 1986; Khan et al., 2013), which results in FA efflux and an increase in nutrient provision for milk production from body stores.

Altering milking frequency during early lactation may modify these homeorhetic mechanisms to support the corresponding change in milk production. Increasing the frequency of milking may exacerbate the negative energy balance, due to the expected increase in milk production and the limitations of nutrient intake, especially in pasture-based dairy systems. The severity

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of the negative energy balance may also depend on the duration of the change in milking frequency. Increasing the milking frequency from twice daily (**2×**) to 3 times daily (**3×**) for 8 wk reportedly increased milk production, but also increased metabolic stress for the duration of the study (Andersen et al., 2004b). However, increasing milking frequency from 2× to either 3× or 4 times daily for 3 or 4 wk immediately postpartum, does not evoke major metabolic effects (McNamara et al., 2008; Soberon et al., 2010).

Increasing milking frequency increases the plasma concentrations of NEFA and BHBA, which indicate increased lipolysis and ketogenesis (Loiselle et al., 2009; Soberon et al., 2010). Furthermore, Eslamizad et al. (2010) reported decreased BCS (a tool for assessing body fat reserves; Roche et al., 2004) and plasma glucose, but no change in hepatic triacylglyceride (**TAG**) concentration, in cows milked 6 times daily compared with cows milked 3×. Additionally, limited data exist on any persistent or carryover effects of increased milking frequency on metabolic indices.

In comparison, decreasing milking frequency may improve energy balance. Cows milked once daily (**1×**) have improved energy balance, reduced BCS loss, and reduced BW loss during early lactation (Patton et al., 2006; McNamara et al., 2008). The metabolic benefits of milking 1× during early lactation are consistent across studies; however, less information is available on metabolic effects of 1× milking once cows are switched to 2×. In a recent study (Kay et al., 2013), decreasing milking frequency from 2× to 1× did not alter plasma GH concentrations; however, glucose, insulin, and IGF-1 concentrations were greater, and BHBA and NEFA concentrations lower, in cows milked 1× compared with 2×. Furthermore, after cows milked 1× were switched to 2× milking, glucose, insulin and IGF-1 concentrations tended to remain greater.

The milk production and BW measures from previous work (Phyn et al., 2011) indicate that metabolic changes occur in response to altered milking frequency and persist even when milked 2×. Therefore, to increase understanding of the homeorhetic mechanisms responsive to milking frequency, plasma concentrations of key hormones and metabolites and transcription of genes involved in the somatotropic axis and lipid metabolism were measured in liver and subcutaneous adipose tissue from cows milked with different frequencies during the period immediately postpartum.

MATERIALS AND METHODS

Experimental Design and Treatments

The study was conducted at Lye Farm, DairyNZ (Hamilton, New Zealand; 37°46′S, 175°18′E) from June to November 2009. All treatments and measurements were approved by the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

The experimental design, grazing management, and supplementary feeding of cows are described in Phyn et al. (2011). Briefly, multiparous Holstein-Friesian and Holstein-Friesian × Jersey dairy cows (n = 150) were randomly allocated to 1 of 5 treatments (n = 30/treatment) immediately postpartum (mean ± SD; July 15, 2009 ± 10.6 d). Treatments were (1) cows milked 2× for the entire lactation (control), (2) cows milked 1× for 3 wk postpartum and 2× thereafter, (3) cows milked 1× for 6 wk postpartum and 2× thereafter, (4) cows milked 3× for 3 wk postpartum and 2× thereafter, and (5) cows milked 3× for 6 wk postpartum and 2× thereafter. Daily milking times were 0700 h for 1× (24-h interval), 0700 and 1500 h for 2× (16/8-h interval), and 0700, 1500, and 2200 h for 3× (9/8/7-h interval).

All cows were offered a generous allowance of perennial ryegrass/white clover pasture (~30 to 45 kg of DM/cow per day measured to ground level with target postgrazing residuals of 1,800 kg of DM/ha). Cows grazed in 1×, 2×, and 3× milking mobs in the same paddock separated by a wire so that they could be drafted independently to the farm dairy for milking. Due to the inherent difficulties of measuring individual DMI under grazing conditions, energy intakes per cow could not be measured, but high postgrazing residuals were targeted to ensure that DMI was not limited by pasture allocation. Furthermore, pasture silage was offered at, on average, 3.6 kg of DM/cow per day for 40 d during July and August to maintain pasture residuals when pasture availability was limited, and pasture quality was maintained by strategic mechanical cutting of high residuals or an immediate regrazing with a nontreatment group of cows. In addition, cows were offered 2 kg of DM/cow per day of pelleted maize/barley/molasses-based concentrate in 1 feed at 0900 h for 2 wk before their expected calving date. Following calving, the same concentrate was individually offered to cows at 4 kg of DM/cow per day during the a.m. milking until November 1 (i.e., ~109 DIM) and at 2 kg of DM/cow per day thereafter, until November 24.

Milk and Blood Sampling and Measurements

Milk production and BCS measurements were as described elsewhere (Phyn et al., 2011). Briefly, individual milk yields and milk composition (fat, CP, and lactose) were determined daily and weekly, respectively. Body condition score (1–10 scale, where 1 = emaciated and 10 = obese; Roche et al., 2004) were measured once per week until September and once every 2 wk thereafter. Blood was sampled on the same 1 d each week for the