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Expression of key lipid metabolism genes in adipose tissue is not altered by once-daily milking during a feed restriction of grazing dairy cows

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ABSTRACT

The objective of this study was to investigate the effect of reduced milking frequency, at 2 feeding levels, on gene expression in adipose tissue of grazing dairy cows during early lactation. Multiparous Holstein-Friesian and Holstein-Friesian × Jersey cows (n = 120) were grazed on pasture and milked twice daily (2×) from calving to 34 ± 6 d in milk (mean ± standard deviation). Cows were then allocated to 1 of 4 treatments in a 2 × 2 factorial arrangement. Treatments consisted of 2 milking frequencies (2× or once daily; 1×) and 2 feeding levels for 3 wk: adequately fed (AF), consuming 14.3 kg of dry matter/cow per day, or underfed (UF), consuming 8.3 kg of dry matter/cow per day. After the treatment period, all cows were fed to target grazing residuals ≥1,600 kg of DM/cow per day and milked 2× for 20 wk. Adipose tissue was collected from 12 cows per treatment by subcutaneous biopsy at −1, 3, and 5 wk relative to treatment start, RNA was extracted, and transcript abundance of genes involved in lipid metabolism was quantified using a linear mixed model. At the end of the 3-wk treatment period, transcript abundance of genes involved in fatty acid (FA) uptake into adipose tissue (*LPL*), FA synthesis [FA synthase (*FASN*) and stearoyl-coenzyme A desaturase (*SCD*)], FA oxidation [acyl-coenzyme A synthetase long-chain family member 1 (*ACSL1*) and carnitine palmitoyltransferase 2 (*CPT2*)], glyceroneogenesis [glycerol-3-phosphate dehydrogenase 1 (*GPD1*) and pyruvate carboxylase (*PC*)], and triacylglyceride synthesis [diacylglycerol O-acyltransferase 2 (*DGAT2*)] were greater in AF1× cows compared with all other treatments. However, when cows were underfed, no effects of milking frequency were observed on transcript abundance of genes involved in adipose lipid metabolism. Despite increases in plasma NEFA concentrations in UF cows, no effects of underfeeding were observed on the transcription of lipolytic genes. At 5 wk, after cows were returned to 2× milking and standard feed allowance, transcript abundances of genes involved in FA synthesis [acetyl-coenzyme A carboxylase α (*ACACA*) and *SCD*)] were increased in cows previously UF. Expression of *ACSL1* was decreased in UF1× cows relative to UF2× cows and *CPT2* expression was greater in AF1× cows compared with AF2× cows. In conclusion, after 3 wk of reduced milking frequency during a feed restriction, transcription of genes involved in lipid metabolism in adipose tissue were not altered, possibly due to the reduced milk production in these animals. However, 3 wk of 1× milking in AF cows increased transcription of genes involved in FA synthesis, oxidation, and triacylglyceride synthesis.
Key words: caloric restriction, adipose tissue, milking frequency, lipostatic theory

INTRODUCTION

Feed shortages can occur in pasture-based dairy systems when the quality or availability of pasture is negatively affected by weather conditions or poor grazing management. When DMI is limited in lactating dairy cows, homeorhetic changes occur to ensure that energy is available to sustain maintenance and milk production (Bauman and Currie, 1980). This can result in a state of negative energy balance and reductions in plasma glucose and insulin concentrations, leading to increased mobilization of adipose tissue reserves for maintenance and milk production (Chilliard et al., 2000).

Lipid mobilization and the increase in plasma NEFA concentrations are the net results of changes to the balance between esterification, lipolysis, and reesterification, as some of the FA released during lipolysis is reesterified to triacylglycerol (**TAG;** Chilliard et al., 2000). Increased NEFA release during a period of negative energy balance is proposed to be a result of decreased reesterification of TAG (Wilson, 1983; Dunshea et al., 1990), and increased lipolysis (Bauman and Currie, 1980) mediated by β-adrenergic receptors (Sumner and McNamara, 2007). Plasma NEFA concentrations plateau or decrease after 4 to 8 d, even with continued

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feed restriction (Vernon, 1992; Gross et al., 2011). This plateau is possibly due to negative feedback to minimize the toxic effects of high NEFA concentrations or to restrict the loss of adipose tissue reserves (Chilliard et al., 2000), as it has been suggested that mammals try to maintain a certain adipose content (Kennedy, 1953) and, in particular, lactating dairy cows (Roche et al., 2008). Managing a cow's energy balance in seasonal dairy systems is crucial during a feed restriction in early or mid-lactation, as negative energy balance can lower milk production and increase the incidence of metabolic disorders.

Reducing milking frequency (**MF**) from twice- (**2×**) to once-daily (**1×**) is one strategy proposed to manage the energy balance of dairy cows (Auldist and Prosser, 1998; Kay et al., 2013), as 1× milking reduces milk production (Davis et al., 1999) and, consequently, decreases energy demands by the mammary gland. Cows milked 1× have reduced BCS and BW loss, and improved energy balance compared with cows milked 2× (Rémond et al., 2002; Guinard-Flament et al., 2007). Milking cows 1× instead of 2× during a feed restriction limits both the increase in plasma NEFA concentrations and the decrease in plasma glucose and insulin concentrations (Guinard-Flament et al., 2007; Kay et al., 2013). However, Kay et al., (2013) reported that the improvements in energy status due to 1× milking were not sufficient to prevent BCS loss during 3 wk of underfeeding of grazing dairy cows during early lactation.

The mechanisms underlying the adipose tissue response to reduced MF during a feed restriction remain to be determined. In lactating goats, the increase in plasma NEFA during a nutrient deficit coincides with changes in adipose tissue gene expression (Faulconnier et al., 2011). Expression of genes involved in hydrolysis of TAG for uptake by adipose tissue [lipoprotein lipase (***LPL***)], FA synthesis [acetyl-CoA carboxylase α (***ACACA***) and stearoyl-CoA desaturase (***SCD***)], glycerol-3-phosphate production [glycerol-3-phosphate dehydrogenase 1 (***GPD1***)], TAG synthesis [glycerol-3-phosphate acyltransferase mitochondrial (***GPAM***)], and FA oxidation [acyl-CoA synthetase long-chain family member 1 (***ACSL1***)] were decreased, whereas the expression of genes involved in FA transport (FA-binding protein 4 (***FAPB4***)] and inhibition of TAG uptake [angiopoietin-like 4 (***ANGPTL4***)] were increased (Faulconnier et al., 2011).

Adipose tissue metabolism during the transition period in cows indicates that lipogenesis is decreased in relation to intake, whereas lipolysis is correlated with milk production (McNamara, 1989); therefore, both aspects may be modified in cows milked 1× during a nutrient deficit. Lipolysis, however, is not consistently accompanied by changes in gene expression, as many of the enzymes are regulated posttranslationally (Sumner and McNamara, 2007; Sumner-Thomson et al., 2011; Khan et al., 2013). These results indicate that transcript abundances of many aspects of lipid metabolism are altered during periods of nutrient deficit; however, it is unclear if 1× milking modifies gene transcription, or whether lipolytic gene expression changes will be measured in grazing cows.

The different effects of nutrition and milk production on lipid metabolism, combined with the lower plasma NEFA concentrations in cows milked 1× relative to 2× (Kay et al., 2013), led to the hypothesis that milking cows 1× during a feed restriction would minimize lipolysis and enhance lipogenesis in adipose tissue. Therefore, the objective of this experiment was to measure the expression of genes involved in lipid metabolism in adipose tissue, during and after a feed restriction in cows milked 1× or 2×, to determine the effect of reduced MF during an energy deficit.

MATERIALS AND METHODS

Experimental Design and Treatments

The study was conducted at the Westpac Taranaki Agriculture Research Station, (Hawera, New Zealand; 39°35′ S 174°17′ E) from July to October 2009. All treatments and measurements were approved by the Ruakura Animal Ethics Committee (Hamilton, New Zealand).

The experimental design, grazing management, milk production, BW, BCS, and plasma hormone and metabolite measurements were as described previously (Kay et al., 2013). Briefly, multiparous Holstein-Friesian and Holstein-Friesian × Jersey dairy cows (n = 120) were grazed on pasture as 1 herd (to target postgrazing residuals of >1,600 kg of DM/ha), and milked 2× for the first 34 ± 6 DIM (mean ± SD). Cows were then allocated to 1 of 4 treatments in a 2 × 2 factorial arrangement. Treatments consisted of 2 MF (1× or 2×) and 2 feeding levels [adequately fed (**AF**) or underfed (**UF**)] and were imposed for 3 wk. At the start of the treatment period, pasture allowances were incrementally increased or decreased over a 3-d period to target an average DMI of 15 or 9 kg of DM/cow per day in the AF and UF treatments, respectively. Daily milking times were 0700 for 1X (24 h milking interval) and 0700 and 1500 for 2X (16/8 h milking interval). To reduce variability due to DIM, cows were grouped into 2 experimental cohorts: cows that calved from July 17 to August 7 (n = 66) were included in cohort 1 and cows that calved from August 8 to September 1 (n = 54) were included in cohort 2. Both cohorts were managed identically. Following the treatment period, all animals