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Hepatic gene expression in multiparous Holstein cows treated with bovine somatotropin and fed n-3 fatty acids in early lactation[1]

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ABSTRACT

Multiparous cows were fed supplemental dietary fat and treated with bST to assess effects of n-3 fatty acid supply, bovine somatotropin (bST), and stage of lactation on hepatic gene expression. Cows were blocked by expected calving date and previous milk yield and assigned randomly to treatment. Supplemental dietary fat was provided from calving as either whole high-oil sunflower seeds (SS; 10% of dietary dry matter; n-6/n-3 ratio of 4.6) as a source of linoleic acid or a mixture of Alifet-High Energy and Alifet-Repro (AF; 3.5 and 1.5% of dietary dry matter, respectively; n-6/n-3 ratio of 2.6) as a source of protected n-3 fatty acids. Cows were treated with 0 (SSN, AFN) or 500 (SSY, AFY) mg of bST every 10 d from 12 to 70 d in milk (DIM) and at 14-d intervals thereafter. Liver biopsies were collected on −12, 10, 24, and 136 DIM for gene expression analysis. Growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP3), hepatic nuclear factor 4α (HNF4α), fibroblast growth factor-21 (FGF-21), and peroxisome proliferator-activated receptor α (PPARα) were the target genes and hypoxanthine phosphoribosyltransferase (HPRT) was used as an endogenous control gene. Expression was measured by quantitative real-time reverse transcription-PCR analyses of 4 samples from each of 32 cows (8 complete blocks). Amounts of hepatic HPRT mRNA were not affected by bST or diet but were increased by approximately 3.8% in early lactation (3.42, 3.52, 3.54, and 3.41×10^4 message copies for −12, 10, 24, and 136 DIM, respectively). This small change had little detectable impact on the ability of HPRT to serve as an internal control gene. Amounts of hepatic GHR, IGF-I, and IGFBP3 mRNA were reduced by 1.5 to 2-fold after calving. Expression of GHR and IGF-I increased and IGFBP3 tended to increase within 12 d (by 24 DIM) of bST administration. These effects of bST persisted through 136 DIM. Hepatic HNF4α mRNA was not altered by DIM or any of the treatments. Abundance of PPARα mRNA was unchanged through 24 DIM but increased by 136 DIM. There was a trend for an interaction of bST, diet, and DIM on PPARα mRNA abundance from 24 to 136 DIM because the amount of PPARα mRNA increased in SSN, SSY, and AFN cows but was not altered in AFY cows. The amount of FGF-21 mRNA increased markedly in early lactation but, like HNF4α mRNA, was not affected by bST, diet, or their interactions. These results indicate 1) that bST induced increases in hepatic expression of GHR, IGF-I, and IGFBP3 when cows were in negative energy balance in early lactation, 2) there was no effect of reduced dietary n-6/n-3 content on hepatic gene expression, and 3) there was support for a potential homeorhetic role of hepatic FGF-21 via uncoupling the somatotropin-IGF-axis in early lactation.
Key words: somatotropin, n-3 fatty acid, hepatic gene expression

INTRODUCTION

The onset of lactation represents an impressive example of homeorhesis because an extensive array of physiological adaptations within multiple tissues occur in a coordinated manner to support the synthesis of large quantities of milk and preserve metabolic homeostasis simultaneously. The transition from pregnant and nonlactating to nonpregnant and lactating can increase nutritional requirements of the high-producing dairy cow by 4-fold. However, these increased requirements are not accompanied by an immediate or sufficient increase in feed intake because feed intake usually decreases at the onset of parturition and the subsequent rate of increase is not as rapid as the increase in milk yield (Bell and Bauman, 1997). Because cows experience a period of significant negative energy balance in early lactation, they must mobilize body tissue to supply the energy and substrates needed to meet the metabolic demands

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for milk synthesis and peripheral needs (Bell and Bauman, 1997). Milk yield and reproductive performance are compromised if the magnitude and duration of this period of insufficient nutrient and energy supply are prolonged.

Efforts to minimize the duration of negative energy balance include the use of supplemental dietary fats. Feeding polyunsaturated fatty acids (**PUFA**) that can escape microbial biohydrogenation in the rumen provides a source of energy, and individual types of PUFA (n-6 vs. n-3, for example) can have differential effects on gene expression and metabolism (Clarke, 2004). Peroxisome proliferator-activated receptors (**PPAR**) are important factors in fatty acid regulation of gene transcription (activation and suppression), and a broad spectrum of fatty acids bind to and activate the primary subtype [proliferator-activated receptor α (**PPARα**)] of this transcription factor (Clarke, 2004). Hepatic nuclear factor 4α (**HNF4α**) is also involved in the regulation of transcription of several enzymes involved in gluconeogenesis and fatty acid metabolism and could be a target for fatty acid control of gene expression (Clarke, 2004). Alterations in the contribution of total PUFA and the ratio of n-6 to n-3 fatty acids have been used in attempts to improve reproduction and immune function and to increase the functional food value of dairy products (Carriquiry et al., 2009a).

The liver plays a critical, central role in the coordinated effort to redirect nutrients toward milk synthesis. Somatotropin (**ST**, or growth hormone) is recognized as a major homeorhetic regulator (Bauman, 2000) and its direct effects are initiated by binding to and activation of the growth hormone receptor (**GHR**). One of the most significant direct effects is stimulation of IGF-I synthesis, which occurs predominantly in the liver (Sjögren et al., 1999). However, in contrast to the typical direct association between ST and IGF-I, circulating ST concentrations begin to increase shortly before parturition and IGF-I concentrations begin to decrease (Radcliff et al., 2003; Rhoads et al., 2004). Decreased expression of GHR, particularly the liver-specific GHR1A transcript, is one of the mechanisms involved in the periparturient decrease in circulating IGF-I (Radcliff et al., 2006) and contributes to results that indicate milk yield and IGF-I response to bST administration are greater in later lactation when cows are in positive energy and nutrient balance (Bauman, 2000). Hepatic fibroblast growth factor-21 (**FGF-21**) is an important regulator of lipid and carbohydrate metabolism (Kharitonenkov et al., 2007), and recent evidence indicates that during fasting, FGF-21 inhibits the signal transducer and activator of transcription 5 signaling pathway downstream of janus kinase 2 (Inagaki et al., 2008. Thus, homeorhetic properties of FGF-21 could be expressed through uncoupling of the ST-IGF axis during the onset of lactation.

We hypothesized that changes in hepatic gene expression associated with the homeorhetic coordination of hepatic adaptation to the onset of lactation would be altered by differences in the dietary n-6 to n-3 fatty acid ratio and by bST administration. Our objectives were to determine effects of initiation of bST administration in early lactation and consumption of dietary fat enriched with n-6 or n-3 fatty acids on hepatic gene expression in periparturient multiparous Holstein cows. We focused on genes associated with the ST-IGF axis and lipid and glucose metabolism.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

Detailed descriptions of the diets, animal management, data and sample collection and analyses, and production responses have been reported elsewhere (Carriquiry et al., 2009a). Briefly, multiparous cows (n = 59) were fed a dry cow diet beginning 3 wk before their expected calving date. Cows were blocked by expected calving date and previous milk yield (305-d mature equivalent) and assigned randomly to 1 of 4 treatments in a 2 × 2 factorial arrangement of bST (0 or 500 mg/injection) and source of supplemental dietary fat. Treatment diets contained either whole high-oil sunflower seeds (10% of dietary DM; **SS**) as a source of linoleic acid (dietary n-6/n-3 ratio = 4.6) or a mixture of Alifet-High Energy and Alifet-Repro (Alifet USA, Cincinnati, OH; 3.5 and 1.5% of dietary DM, respectively; **AF**) as a source of n-3 fatty acids (dietary n-6/n-3 ratio = 2.6). Alifet-High Energy is a microcrystallized rumen-inert energy concentrate made from animal fat (99%) rich in SFA (57% stearic acid; 25% palmitic acid). Alifet-Repro is a microcrystallized rumen-inert fat (flaxseed oil and fish oil) that is enriched with the n-3 fatty acids linolenic (C18:3, 15.7%), eicosapentaenoic (C20:5, 1.3%), and docosahexaenoic (C22:6, 1.3%) acids. Dietary n-6/n-3 was calculated as the ratio of (18:2 *cis*-9, *cis*-12) to (18:3 *cis*-9, *cis*-12, *cis* 15 + C20:5 + C22:6). The SS and AF diets contained 383 and 265 mg of 18:2 *cis*-9, *cis*-12 per gram of total fatty acid, which would provide 756 and 463 g of 18:2 *cis*-9, *cis*-12 for cows that consumed 25 kg of DM/d, respectively (Carriquiry et al., 2009b).

Diets were fed as TMR and were formulated to meet nutritional needs (NRC, 2001) of the cows. Diets were composed primarily of alfalfa haylage, corn silage, high-moisture shelled corn, and soybean meal. The dry cow diet contained 17.7% CP and 1.70 Mcal of $NE_{L\text{-}Actual\ DMI}$. Treatment diets were designed to differ only in the type of fatty acids they contained and were formulated to