J. Dairy Sci. 95:7097–7104
http://dx.doi.org/10.3168/jds.2012-5613

Bovine hepatic and adipose retinol-binding protein gene expression and relationship with tumor necrosis factor-α

P. Rezamand,*[1] J. S. Watts,* K. M. Hunt,* B. J. Bradford,† L. K. Mamedova,† and S. D. Morey†
*Department of Animal and Veterinary Science, University of Idaho, Moscow 83844
†Department of Animal Science and Industry, Kansas State University, Manhattan 66506

ABSTRACT

Retinol-binding protein (RBP) is the main transport system for retinol in circulation, is a relatively small protein with one binding site for retinol in the *all-trans* form, and is bound to transthyretin. The objectives of this study were to characterize the temporal pattern of bovine hepatic mRNA expression of *RBP* during the periparturient period and to determine if a relationship exists between the expression of *RBP* and that of tumor necrosis factor (*TNF*)-α in dairy cows. In experiment 1, we assessed hepatic mRNA expression of *RBP* during the periparturient period. Liver tissues were sampled from periparturient dairy cows (n = 9) at −21, −4, +1, +7, and +21 relative to parturition and frozen in liquid N_2. Total RNA was extracted from each tissue sample and cDNA was generated. Gene expressions of *RBP* and β-actin (as a housekeeping gene) were measured as relative quantity using reverse transcription-PCR. Data were analyzed using cycle threshold values, adjusted to β-actin, and significance was determined at $P < 0.05$. Serum samples (−21, −4, +1, +7, and +21 relative to parturition) were analyzed for retinol concentration using a standard HPLC-based method. Cows had variable expression of hepatic *RBP* and serum retinol over the transition period, with a decline near parturition and a rebound toward prepartum levels later in lactation. In experiment 2, liver and visceral (intestinal) adipose tissues were sampled from dairy cows (n = 28) at slaughter. Expression of *RBP* and *TNF-α* was detected in all samples and variations among cows were highly significant for both genes. Across tissues, expression of *RBP* was positively correlated with that of *TNF-α* (r = 0.60). Within adipose tissue, expression of *RBP* and *TNF-α* was weakly correlated (r = 0.23), whereas in hepatic tissue, expression was strongly correlated (r = 0.62). In experiment 3, late-lactation dairy Holstein cows were blocked by parity and feed intake, and randomly assigned to control, recombinant bovine (rb)TNF challenge, or pair-fed control treatment (n = 5/treatment). Cows were injected with either rbTNF (subcutaneous injection of 2 μg/kg of body weight in saline) or sterile saline (control and pair-fed control animals) once daily for 7 d. Liver biopsy was performed on d 7 and samples were processed for expression of *RBP* and *TNF-α*. Although TNF challenge caused an upregulation of hepatic *TNF-α* expression, as expected, it did not alter hepatic *RBP* expression. Overall, the temporal pattern of hepatic *RBP* gene expression during the periparturient period followed, to a great extent, that of plasma retinol. Although a strong positive correlation was previously detected between bovine hepatic *RBP* and *TNF-α* transcripts, rbTNF challenge did not cause alter *RBP* expression. These observations collectively imply that regulation of RBP at the transcription level is influenced by physiological state but may be independent from that of transthyretin, which is altered by proinflammatory stimuli (such as TNF-α) via induction of transcription factor nuclear factor-interleukin 6.

Key words: gene expression, retinol-binding protein, tumor necrosis factor

INTRODUCTION

Retinol-binding protein (**RBP**) is the main transport system for retinol in circulation (Heller, 1975a). Circulating RBP (also called RBP4) is a relatively small protein (21 kDa) with one binding site for retinol in the *all-trans* form, is bound to transthyretin (**TTR**), and is primarily synthesized in the liver (Heller, 1975b Nonnecke et al., 2001). Extra-hepatic sources of RBP have also been identified and characterized (Liu et al., 1990; Liu and Godkin, 1992). Furthermore, plasma RBP and TTR concentrations are reduced in association with metabolic disorders such as hyperketonemia (Gröhn and Lindberg, 1985). Alterations in RBP concentrations and other hepatic export proteins such as haptoglobin have been used as indicators of immune response to inflammation and are part of the acute phase response (Humblet et al., 2006). Indeed, plasma RBP and TTR concentrations were both reduced during an

Received April 11, 2012.
Accepted August 17, 2012.
[1]Corresponding author: rezamand@uidaho.edu

inflammation caused by LPS, an observation related to induction of the transcription factor nuclear factor IL-6 (**NF-IL6**) by elevated proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-α (**TNF-α**; Rosales et al., 1996).

Abd Eldaim et al. (2010) demonstrated that plasma RBP concentrations fluctuated as dairy cows went through the transition period, with a sharp decline immediately postpartum and a rebound toward prepartum levels later in lactation. Because RBP was detected at high concentrations in colostrum but scarcely in milk, the authors (Abd Eldaim et al., 2010) attributed the observed sharp decline in plasma RBP concentrations to its flux toward the mammary glands at parturition and during the days immediately following parturition. In another study, we reported that occurrence of a new IMI was associated with a significant reduction in plasma RBP at wk 1 postpartum that was not associated with a decrease in plasma retinol, which would be expected because RBP is the main transporter for retinol (Rezamand et al., 2007). Induction of transcription factor NF-IL6 by proinflammatory cytokines such as TNF-α may result in downregulation of the hepatic synthesis of acute phase proteins such as TTR. It is unknown, however, whether this downregulation affects RBP status.

We hypothesized that a tissue-specific association exists between expression of *RBP* and that of *TNF-α* in bovine hepatic and adipose tissues, and that the relationship between the transcripts would be of a causal nature. Our specific objectives were to determine (1) the temporal pattern of hepatic mRNA expression of RBP during the periparturient period, (2) whether a relationship exists between hepatic and adipose mRNA expressions of *RBP* and that of a key inflammatory mediator, *TNF-α*, and (3) if the relation between *RBP* and *TNF-α* is of a causal nature at the transcription level.

MATERIALS AND METHODS

Experiment 1: Periparturient Cows

Nine Holstein dairy cows were monitored from 21 d before expected calving date through 21 d postpartum. Animals were housed in a tie-stall facility, milked 3 times daily (0400, 1100, and 2100 h) postpartum, and fed twice daily (0700 and 1500 h) to ensure approximately 10% orts. Prepartum and postpartum diets were formulated to meet requirements (NRC, 2001). The prepartum diet (63.8% DM) included corn silage, prairie hay, wet corn gluten feed, ground corn, solvent-extracted soybean meal, salt, and mineral/vitamin premix, and contained (on a DM basis) 13.8% CP, 41.3% NDF, 17.9% starch, 2.4% ether extract, and 7% ash. The lactation ration (60.9% DM) included corn silage, alfalfa hay, wet corn gluten feed, ground corn, mechanically extracted soybean meal, salt, and mineral/vitamin premix, and contained (on a DM basis) 18.9% CP, 27.9% NDF, 22.7% starch, 2.9% ether extract, and 8.2% ash.

Liver samples were obtained on d −21, −4, +1, +7, and +21 relative to expected parturition date at 1300 h, as described (Morey et al., 2011). Briefly, liver samples were collected using a 14-gauge × 15-cm biopsy needle (SABD-1415-15-T, US Biopsy, Franklin, IN). Liver tissue was collected between the 10th and 11th ribs, 5 cm dorsal to a line between the olecranon and tuber coxae. The area was shaved, aseptically prepared, and anesthetized with 2 mL of subcutaneous lidocaine hydrochloride. Anesthesia was assessed by cutaneous response after 5 min, and a #11 Bard Parker blade was used to make a stab incision into the body wall. The biopsy needle was inserted cranioventrally toward the liver and a total of approximately 100 mg of tissue was collected (approximately 20 mg each time), frozen in liquid nitrogen, and stored at −80°C until analysis. The mRNA abundance of RBP in liver tissue was determined by real-time PCR. Briefly, RNA was extracted from tissue homogenate using the RNeasy Lipid Tissue Mini Kit (Qiagen Inc., Valencia, CA). Purity and concentration of total RNA were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Rockland, DE). Complementary DNA was then synthesized from 2 μg of total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Primers for PCR amplification of *RBP* and β-actin (***BAC***) were designed by Primer Express (version 1.5, Applied Biosystems), generating the primers detailed in Table 1. The reaction was carried out at 50°C for 2 min, followed by a denaturing step at 95°C for 10 min, and then 40 cycles of 95°C and 60°C.

Gene expression of *RBP* and *BAC* was measured using the 7500 Fast Real-Time PCR System (Applied Biosystems) as previously reported (Shields et al., 2011). Reactions included 2 μL of cDNA, 10 μL of Applied Biosystems Taqman Universal PCR Mastermix (Applied Biosystems), 1 μL of Applied Biosystems 20× custom primer probe mixture, and 7 μL of water.

Plasma retinol concentration was determined by reversed-phase HPLC (Waters e2695 Separation Module, Waters Corp., Milford, MA) with a photodiode array detector (Waters 2998, Waters Corp.). Briefly, 400-μL plasma samples were mixed with 400 μL of methanol + 20 μL of acetic acid, and 50 μL of retinol acetate (Sigma-Aldrich, St. Louis, MO) as the internal standard. Samples were extracted once with 1.8 mL of a