J. Dairy Sci. 97:330–339
http://dx.doi.org/10.3168/jds.2013-7222

Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows

M. Zarrin,*†‡ O. Wellnitz,* H. A. van Dorland,*[1] and R. M. Bruckmaier*[2]
*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bremgartenstrasse 109a, 3001 Bern, Switzerland
†Department of Animal Science, Agriculture Faculty, Yasouj University, Student Street, 75918-74831, Yasouj, Iran
‡Graduate School for Cellular and Biomedical Sciences, University of Bern, Freiestrasse 1, 3012 Bern, Switzerland

ABSTRACT

Metabolic adaptations during negative energy and nutrient balance in dairy cows are thought to cause impaired immune function and hence increased risk of infectious diseases, including mastitis. Characteristic adaptations mostly occurring in early lactation are an elevation of plasma ketone bodies and free fatty acids (nonesterified fatty acids, NEFA) and diminished glucose concentration. The aim of this study was to investigate effects of elevated plasma β-hydroxybutyrate (BHBA) at simultaneously even or positive energy balance and thus normal plasma NEFA and glucose on factors related to the immune system in liver and mammary gland of dairy cows. In addition, we investigated the effect of elevated plasma BHBA and intramammary lipopolysaccharide (LPS) challenge on the mammary immune response. Thirteen dairy cows were infused either with BHBA (HyperB, n = 5) to induce hyperketonemia (1.7 mmol/L) or with a 0.9% saline solution (NaCl, n = 8) for 56 h. Two udder quarters were injected with 200 μg of LPS after 48 h of infusion. Rectal temperature (RT) and somatic cell counts (SCC) were measured before, at 48 h after the start of infusions, and hourly during the LPS challenge. The mRNA abundance of factors related to the immune system was measured in hepatic and mammary tissue biopsies 1 wk before and 48 h after the start of the infusion, and additionally in mammary tissue at 56 h of infusion (8 h after LPS administration). At 48 h of infusion in HyperB, the mRNA abundance of serum amyloid A (SAA) in the mammary gland was increased and that of haptoglobin (Hp) tended to be increased. Rectal temperature, SCC, and mRNA abundance of candidate genes in the liver were not affected by the BHBA infusion until 48 h. During the following LPS challenge, RT and SCC increased in both groups. However, SCC increased less in HyperB than in NaCl. Quarters infused with LPS showed a more pronounced increase of mRNA abundance of IL-8 and IL-10 in HyperB than in NaCl. The results demonstrate that an increase of plasma BHBA upregulates acute phase proteins in the mammary gland. In response to intramammary LPS challenge, elevated BHBA diminishes the influx of leukocytes from blood into milk, perhaps by via modified cytokine synthesis. Results indicate that increased ketone body plasma concentrations may play a crucial role in the higher mastitis susceptibility in early lactation.
Key words: hyperketonemia, immune response, lipopolysaccharide, dairy cow

INTRODUCTION

For several decades, dairy cows have been selected for high milk production. With the increase in milk production, the incidence of infectious diseases has increased (Syväjärvi et al., 1986; Simianer et al., 1991; Uribe et al., 1995). Mainly during the first weeks of lactation, cows experience immunosuppression and have a high susceptibility to infectious diseases (Smith et al., 1985; Hogan et al., 1989; Goff, 2006), which is thought to be due to the metabolic adaptations to negative energy balance (**NEB**; Suriyasathaporn et al., 2000; van Dorland et al., 2009). Low plasma glucose concentrations and elevated plasma NEFA and ketone body concentrations, specifically BHBA, are the characteristic changes during NEB (Bobe et al., 2004; van Dorland et al., 2009; Gross et al., 2011). Increased plasma BHBA concentrations above 1,200 μmol/L are considered indicative of a metabolic disorder (Ospina et al., 2010) and defined as subclinical ketosis (Duffield et al., 2009).

The occurrence of mastitis is determined by several pathogen, animal, and environmental factors (Burvenich et al., 2007). Among the animal factors, high plasma BHBA concentration has a negative influence on the susceptibility of mastitis and course of disease (Heyneman et al., 1990; Oltenacu and Ekesbo, 1994; Van Werven et al., 1997). In vitro, growth of bacteria

Received July 2, 2013.
Accepted September 25, 2013.
[1]Present address: School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Laenggasse 85, 3052 Zollikofen, Switzerland.
[2]Corresponding author: rupert.bruckmaier@vetsuisse.unibe.ch

obtained from LPS-challenged quarters in hyperketotic and normal dairy cows showed that high plasma BHBA concentrations had a high positive correlation with the severity of mastitis induced by *Escherichia coli* (Kremer et al., 1993). In addition, elevation of plasma BHBA concentration decreased in vitro chemotaxis and microbial killing in human neutrophils (McMurray et al., 1990) and respiratory burst activity of bovine neutrophils (Hoeben et al., 1997). Cytokine production was reduced after bacterial infection in ketotic dairy cows (Filar et al., 1992; Kandefer-Szerszen et al., 1992). However, elevated BHBA occurs mostly concomitantly with other metabolic and endocrine changes (Kessel et al., 2008; Gross et al., 2011), and the immunosuppressive effect cannot be exclusively ascribed to the ketone bodies. Results of exclusive BHBA effects on the immune system are available from in vitro studies. Thus, in the presence of BHBA, a decreased phagocytotic activity of milk polymorphonuclear leukocytes was demonstrated (Kluciński et al., 1988), as well as reduced chemotactic capacity of bovine blood leukocytes (Suriyasathaporn et al., 1999). The role of BHBA in immunosuppression in vivo is not fully understood (Burton et al., 2005). Information about the effects of hyperketonemia isolated from other metabolic changes on the immune response and SCC in hyperketotic cows is rare, and the effects of long-term hyperketonemia have not been investigated in dairy cows. The objective of this work was to investigate effects of a 48-h elevation of plasma BHBA, through BHBA infusion, on parameters related to the immune system in liver and mammary gland, and to study the mammary immune response to LPS challenge during elevated BHBA for additional 8 h.

MATERIALS AND METHODS

Animals and Management

All procedures involving animals followed the Swiss Animal Protection Law and were approved by the Federal Veterinary Office in Switzerland. Thirteen nonpregnant Holstein dairy cows in diestrus with a parity of 3.5 ± 0.10, at 28 ± 0.3 (mean ± SD) wk in milk were selected. Cows in mid lactation were chosen as the best possible model for investigation of the specific effect of BHBA infusion without the comprehensive endocrine and metabolic changes that occur during the period of highest milk production in early lactation. The cows were free of mastitis and other infectious diseases, which was confirmed by a routine blood glutaraldehyde coagulation test (Sandholm, 1976), and by measuring milk SCC (DeLaval cell counter DCC, DeLaval International AB, Tumba, Sweden), which had to be $<150 \times 10^3$ cells/mL in all 4 quarters at the start of the experiment. The validity and repeatability of the DeLaval cell counter was previously shown (Sarikaya and Bruckmaier, 2006).

Animals were moved to the experimental tiestall for adaptation to housing and feeding conditions 2 wk before the start of the experiment. Both groups were fed ad libitum with hay (DM content, 890 g/kg of fresh matter; on DM basis, consisting of 153 g of CP/kg, 235.0 g of crude fiber/kg, and 5.7 MJ of NE_L/kg). In addition, cows received a protein- and energy-rich concentrate (23.5% barley, 14.0% oats, 20.0% wheat bran, 17.0% soybean expeller, 15.0% linseed meal, 0.6% salt, 2.2% carbonate of lime, 0.4% calf rearing feed premix, 4.0% molasses, and 3.0% bypass fat, DM content, 881 g/kg of fresh matter; on DM basis, consisting of 217 g of CP/kg, 73.9 g of crude fiber/kg, and 7.6 MJ of NE_L/kg) twice daily according to individual milk production. In addition, minerals (50 g/cow) were supplied once a day. Animals had access to fresh water ad libitum. Milking was performed twice daily at 0530 and 1600 h.

Experimental Design and Treatments

Cows were randomly allocated to 1 of 2 infusion treatment groups, as described earlier (Zarrin et al., 2013). In brief, the treatments involved an intravenous Na-DL-β-OH-butyrate infusion to obtain a plasma BHBA concentration between 1.5 and 2.0 mmol/L (**HyperB**, n = 5), comparable to those in spontaneous hyperketonemia, and a saline (0.9%) infusion (NaCl, 20 mL/h, n = 8) as the control group. One day before the infusions started, cows were fitted with indwelling intravenous catheters (16-gauge; Cavafix Certo Splittocan, B. Braun Melsung AG, Melsungen, Germany) with a length of 32 cm in both jugular veins. The infusions started at 0900 h on d 1 and continued until 1700 h 2 d later. After 48 h of infusions, each of 2 udder quarters was injected with 200 μg of LPS *Escherichia coli* serotype 026:B6 (#L8274; Sigma-Aldrich, St. Louis, MO) as LPS quarters, and 0.9% NaCl solution were injected in 2 udder quarters as control. Details were described by Vernay et al. (2012).

Data Collection and Sampling

Blood Samples. Baseline blood samples at 1 wk and at 2 h before the start of the infusion were taken between milking and feeding at 0730 h. Sampling was continued at least hourly during the 56-h infusion period. During the experimental infusions, blood samples were obtained from the contralateral jugular catheter, which was not used for infusion, using tubes containing tri-potassium-EDTA. Plasma was separated by centrifugation at 3,000 × *g* for 20 min, and stored at −20°C