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Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows

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ABSTRACT

The objectives were to study the effects of induced subclinical hypocalcemia [SCH, blood ionized Ca (iCa^{2+}) <1.0 m*M*, without recumbency] on physiological responses and function of immune cells in dairy cows. Ten nonpregnant, nonlactating Holstein cows were blocked by lactation and assigned randomly to a normocalcemic (NC; intravenous infusion of 0.9% NaCl i.v. plus 43 g of oral Ca, as Ca sulfate and Ca chloride, at −1 and 11 h) or an induced SCH [SCHI, 5% ethylene glycol tetraacetic acid (EGTA), a selective iCa^{2+} chelator, intravenous infusion] treatment for 24 h, using a crossover design. The sequence of treatments was either NC–SCHI or SCHI–NC, with a 6-d washout period. Ionized Ca was evaluated before, hourly during the infusion period, and at 48 and 72 h, to monitor concentrations and adjust the rate of infusion, maintaining blood iCa^{2+} <1.0 m*M* in SCHI throughout the 24-h infusion period. Additional measurements included heart and respiratory rates, rectal temperature, dry matter intake, rumen contractions, whole-blood pH, concentrations of glucose and K in whole blood, concentrations of total Ca, Mg, nonesterified fatty acids, β-hydroxybutyrate, and insulin in plasma, and urinary excretion of Ca. Total and differential leukocyte count in blood was also performed. The concentration of cytosolic iCa^{2+} in neutrophils and lymphocytes was quantified and neutrophil function was assayed in vitro. Infusion of a 5% EGTA solution successfully induced SCH in all SCHI cows, resulting in decreased blood iCa^{2+} concentrations throughout the 24-h treatment period (0.77 ± 0.01 vs. 1.26 ± 0.01 m*M* iCa^{2+}). Induction of SCH reduced dry matter intake on the day of infusion (5.3 ± 0.8 vs. 9.1 ± 0.8 kg/d) and rumen contractions (1.9 ± 0.2 vs. 2.7 ± 0.2 contractions/2 min) for the last 12 h of infusion. Cows in SCHI had decreased plasma insulin concentration (1.44 ± 0.23 vs. 2.32 ± 0.23 ng/mL) evident between 6 and 18 h after the beginning of the infusion, accompanied by increased concentrations of glucose (4.40 ± 0.04 vs. 4.17 ± 0.04 m*M*). Plasma nonesterified fatty acids concentration was greater for SCHI than NC cows (0.110 ± 0.019 vs. 0.061 ± 0.014 m*M*). Neutrophils of cows in SCHI had a faster decrease in cytosolic iCa^{2+} after stimulation with ionomycin (9.9 ± 1.0 vs. 13.6 ± 1.4 Fluo-4:Fura Red post-end ratio) in vitro. Furthermore, induction of SCH reduced the percentage of neutrophils undergoing phagocytosis (22.1 ± 2.1 vs. 29.3 ± 2.1%) and reduced the oxidative burst response after incubation of pathogenic bacteria (16.1 ± 1.7 vs. 24.2 ± 1.7%). Subclinical hypocalcemia compromised appetite, altered metabolism, and impaired function of immune cells in dairy cows.

Key words: dairy cow, immune function, metabolism, subclinical hypocalcemia

INTRODUCTION

Dairy cows have between 2 and 4 g of Ca in blood, half of which is in the ionized form (**iCa^{2+}**). On the first day of lactation, synthesis and secretion of colostrum impose major losses of Ca equivalent to 7 to 10 times the amount of Ca present in blood (Horst et al., 2005). To cope with this rapid Ca loss and maintain normocalcemia, homeostatic mechanisms have to be in place to increase the influx of Ca into the blood (Goff, 2008). However, the inability of the cow to fully reestablish concentrations of Ca in blood, through intestinal absorption or bone resorption, likely explain the high prevalence of subclinical hypocalcemia (**SCH**) in primiparous (25%) and multiparous (47%) cows (Reinhardt et al., 2011). Change in cytosolic iCa^{2+} is a component of important cellular messenger systems, and increased cytosolic iCa^{2+} concentration is required for regulation of important processes such as neurotransmission, muscle contraction, cell metabolism, cell growth, cell proliferation, and activation of immune cells, among others (Saris and Carafoli, 2005; Parekh, 2006; Vig and Kinet, 2009). Cows with milk fever are at increased risk of developing other periparturient

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problems, including dystocia and ketosis (Curtis et al., 1983), displaced abomasum (Massey et al., 1993), uterine prolapse (Risco et al., 1984), and retained placenta (Melendez et al., 2004). The increased risk of developing these conditions is likely mediated by several factors, including the effects of low iCa^{2+} suppressing smooth muscle contractions (Hansen et al., 2003). Furthermore, hypocalcemic cows have increased plasma concentrations of cortisol (Horst and Jorgensen, 1982), reduced proportion of neutrophils with phagocytic activity (Ducusin et al., 2003; Martinez et al., 2012), and impaired mononuclear cell response to an antigen-activating stimulus (Kimura et al., 2006). This reduction of immune response has linked hypocalcemia to infectious diseases of bacterial origin such as metritis (Martinez et al., 2012) and mastitis (Curtis et al., 1983).

Although the consequences of SCH have not been fully established, affected cows during the first 3 DIM had marked increases in lipid mobilization and a decreased percentage of neutrophils with phagocytic and killing activities, and were at much greater risk of developing metritis and puerperal metritis compared with normocalcemic cows (Martinez et al., 2012). In corroboration with these findings, lactating grazing cows with SCH in the first 7 DIM had increased incidence of metritis and endometritis, along with higher concentrations of NEFA and BHBA in plasma (Ribeiro et al., 2013). Descriptive data from cows induced to have SCH indicated depressed feed intake and rumination (Hansen et al., 2003). Collectively, these studies suggest that SCH is associated with immune dysfunction, exacerbated negative nutrient balance, and greater incidence of uterine diseases in early postpartum dairy cows. Despite the strong link between SCH and peripartum diseases, most studies have been observational in nature and performed during the early postpartum period, when it is difficult to isolate the effect of inadequate concentrations of Ca and iCa^{2+} in blood from the combined metabolic and endocrine disturbances that might influence immune function.

The hypotheses of the current study were that SCHI, defined as blood iCa^{2+} concentration <1.0 m*M* without recumbency, would reduce DMI and compromise measures of energy metabolism, and reduce cytosolic concentrations of iCa^{2+} in leukocytes, thereby depressing measures of white blood cell function in dairy cows. Therefore, the objectives were to study the effects of SCHI on physiological responses and function of immune cells in dairy cows.

MATERIALS AND METHODS

All procedures involving cows in the study were approved by the University of Florida Institutional Animal Care and Use Committee and by the University of Florida Institute of Food and Agricultural Sciences Animal Research Committee.

Cows and Housing

Ten nonpregnant, nonlactating Holstein cows from the University of Florida Dairy Unit (Gainesville) were enrolled in the study. The mean (± SD) age, lactation number, and BW at enrollment were, respectively, 4.4 ± 0.4 yr, 2.4 ± 0.3 lactations, and 696.8 ± 29.2 kg. Selection criteria involved being healthy to a physical exam at the time of enrollment and no history of disease within the last 30 d. Cows were dried off at least 1 wk before the beginning of the study. Cows were moved to individual stalls in the Large Animal Clinic of the Veterinary Teaching Hospital at the University of Florida (Gainesville) at least 4 d before initiating the study to acclimate to the experimental facilities.

Intravenous Solutions

A sterile commercial solution containing 0.9% NaCl (Hospira Inc., Lake Forest, IL) was used. Ethylene glycol-bis (2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (**EGTA**, cat. no. E3889, Sigma Aldrich, St. Louis, MO) 5% solution was prepared aseptically under a laminar flow hood using 900 mL of sterile water (Baxter Healthcare Corp., Deerfield, IL), 50 g of EGTA, and 50 mL of 5 *M* NaOH. The solution was continuously mixed using a vortex until salts were fully dissolved and pH was measured. Additional 5 *M* NaOH solution was added until the pH was adjusted to 7.4. The fully dissolved solution was then filtered using a 0.45-μm bacteriological filter. The solution was then labeled and stored at 4°C until use within 10 d.

Experimental Design and Treatments

The study followed a crossover design in which the 2 treatments were applied to each cow in 2 different periods. Cohorts of cows were blocked based on lactation number and, within each block, cows were assigned randomly to 1 of 2 treatment sequences. The treatments were (control) normocalcemia (**NC**) or induced subclinical hypocalcemia (**SCHI**). Therefore, the sequence of treatments for a given cow was either NC–SCHI or SCHI–NC. Treatments were administered in sequence with a 6-d washout period between treatment administrations to minimize carryover effects.

The day of intravenous infusion was considered study d 0. On study d −1, all cows had the jugular groove area shaved and surgically disinfected, and an intravenous indwelling catheter (14-gauge, 140 mm, Abbocath-T,